STUDIES ON THE GENUS
ARTHROBOTRYS

by

KAREN KAYE HAARD

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Department of Botany and Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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INTRODUCTION

The genus Arthrobotrys Corda is found amid the predaceous Hyphomycetes of the Fungi Imperfecti. It is a small genus presently containing some twenty species some of which are apparently distributed worldwide. Most of the known species have been found in the United States, Great Britain, and the U.S.S.R., but occasional species have also been reported from Denmark, France, India, Algiers, the Belgium Congo, and Argentina.

Most, if not all of the species of this genus are predators of nematodes and springtails (Collembola), a characteristic which makes the biology of these organisms an extremely interesting subject. This feature is not unique with Arthrobotrys, however. There are some twenty-four other genera that exhibit some sort of predaceous activity on nematodes, rotifers, and amoebae. These are divided mainly among the Zoopagaceae and the Hyphomycetes with a few genera scattered elsewhere.

There are eight genera within the Hyphomycetes that attack nematodes or other such "host" organisms. Three of these genera, Arthrobotrys, Dactylella Grove, and Dactylaria Sacc., are primarily saprophytes. Just how important the "host" organisms are to the overall welfare of these fungi is not known. These three genera produce snares or adhesive trapping mechanisms that hold the "host" organism while penetrating hyphae invade it and absorb food material from it. However, the genera Trichothecium Link, Acrostalagmus Corda, Harposporium Lohde., Meria Vuill., and Nematoctonus Drechsler, are actually parasitic on their host organisms. Their spores attach to the host organism and form hyphae within it which later produces conidiophores on the host's integument. Although the species of Arthrobotrys have received considerable attention in regard to their predaceous activity, little has been done with them taxonomically

and thus a comprehensive treatment of this genus is greatly needed. It is hoped that the present study will help in this regard. Special attention has been paid to the development of the conidia and conidiophores since these furnish the main characteristics that distinguish this genus from other genera. Eight of the twenty species in this genus have been studied in both pure and nematode infested cultures. Two keys to twelve of the species are presented for the first time. One key is to species growing in pure culture while the other is to the same species growing in nematode infested culture. These keys include all species believed to be valid. It is hoped that this study will stimulate other workers to isolate and study these interesting organisms.

LITERATURE REVIEW

The literature pertaining to the genus Arthrobotrys may be divided into two parts. One consists of taxonomic papers while the other consists of papers of biological and morphological importance. For purposes of ease and clarity, these two parts are treated separately.

Taxonomic Studies

The genus Arthrobotrys was described by Corda(1839) on the basis of A. superba Corda which became the type species. Corda recognized most of the important characteristics that are used today. Perhaps the single most important feature that he noted was the fact that the conidia were formed on sterigmata in a whorled pattern at the tip and on the nodes of the conidiophores. He also described the conidiophores to be simple, erect, and septate, and the conidia to be two-celled and to have an apiculiform base.

The genus Arthrobotrys was distinguished from the genus Trichothecium Link

with which it was often confused in a publication by Hoffman(1854). He pointed out that although both of these genera had single, simple, erect conidiophores, *Trichothecium* did not bear its conidia in a whorled arrangment as did *Arthrobotrys*. However, even after Hoffman's work, many investigators still confused these two genera. Much of this later confusion was discussed and clarified by Drechsler(1937).

The second species, *A. oligospora*, described by Fresenius in 1850, differed from *A. superba* only in the size and shape of conidia. *A. oligospora* was described as having pyriform conidia while *A. superba* had ovoid conidia.

When Coemans(1863) revised the genus *Arthrobotrys*, he decided from a study of living material that *A. superba* was the only valid species. He divided it into three varieties. The first, "variety *superba*", was distinguished by its highly nodular conidiophores and its pyriform conidia. A second, "variety *oligospora*", had few nodes but it also had pyriform conidia. The third variety, which was not named, was distinguished from the others by its branched conidiophores.

Matruchot(1892) reviewed the previous studies done on the genus and decided from a study of the literature that *A. oligospora* and *A. superba* were in actuality separate species. Matruchot did some interesting studies on *A. oligospora*. He grew it in pure culture on carrots and varied the temperature and humidity in an unsuccessful attempt to induce it to form perithecia. He also studied the mycelium and found that it anastomosed frequently and often formed monocellular, round, nonpedicellate chlamydospores.

The identity of *A. superba* and *A. oligospora* as separate species was finally clarified and firmly established by Drechsler(1937). He studied material on the two species and concluded, in agreement with Fresenius and Matruchot, that the two differed in both conidium size and shape and in the

formation of chlamydospores. He found that *A. oligospora* formed chlamydospores while *A. superba* did not. He also discovered that *A. superba* formed predaceous networks and he verified Zopf's(1888) observation that *A. oligospora* did also.

The third species described in the genus *Arthrobotrys* was described by Preuss(1851) as *A. recta* Pr. The original description did not contain enough information to be of importance taxonomically. Neither Coemans(1863) nor Matruchot(1892) were able to find available material to study although Coemans did state that material was deposited in the Klotze herbarium. Despite this, Matruchot suggested that because of its obovoid spores, *A. recta* might be synonymous with *A. oligospora*, although he added that the description was too brief to base any conclusions.

Preuss(1853) also described *A. longispora*, but he again gave a description too brief to be of importance. He did, however, describe the conidiophores as being partly branched. Coemans(1863) considered this species among the varieties of *A. superba*. It is possible that he was using this species when he described his third variety, which he distinguished only by its branched conidiophores.

Matruchot(1892) again considered the information in Preuss' description of *A. longispora* to be of little or no value because of it's briefness. However, this species was redescribed by Soprunov(1958) as having nodular unbranched conidiophores, obconical conidia and predaceous networks on the basis of material that he had studied.

The total number of species of *Arthrobotrys* was brought to five when Massee described *A. rosea* Massee(1885). This species was distinguished only by its obovoid spores. All other important taxonomic data was omitted from the description. From a study of the literature, Matruchot(1892) concluded

that this species was synonymous with A. oligospora. Drechsler(1937), after a review of the literature, was also of this opinion.

All five of these previously described species were included in Saccardo's Syllogie Fungorum(1886).

Another member of the genus Arthrobotrys was first described by Berlese(1888) as Cephalothecium roseum Corda var. arthrobotryoides Berlese. Berlese apparently confused the two genera as his description of this species was brief but accurate. The nodular conidiophore and whorled arrangment of conidia indicated a close relationship of this fungus to A. superba and A. oligospora, however, the shape of the conidia distinguished it from them.

Matruchot(1892), after a study of the literature, transferred Cephalothecium roseum var. arthrobotryoides to the genus Arthrobotrys as a synonym of A. oligospora. He concluded that it was a synonym because it had a nodular conidiophore development and ovoid to obovoid conidia.

Matruchot(1892) also studied a species that he called A. superba Corda var. irregularis Matr. This variety of A. superba differed from Corda's in that it was branched and nodular. Corda's species was not branched.

Lindau(1907) apparently did not recognize Matruchot's(1892) work as he raised Cephalothecium roseum var. arthrobotryoides to species rank in the genus Arthrobotrys. He listed it as Arthrobotrys arthrobotryoides Lindau giving Cephalothecium roseum var arthrobotryoides as a synonym but he gave no reasons for doing so.

Drechsler(1937) studied A. arthrobotryoides and decided to follow Lindau's (1907) classification. Drechsler noted in his studies that this species did not always have localized nodes but that the conidia were often born on sterigmata along an irregularly branched rachis.

Drechsler was also familiar with Matruchot's(1892) work. He concluded from Matruchot's description of A. superba var. irregularis that this isolate may have actually been A. arthrobotryoides as it had both irregularly spaced nodes and branched conidiophores.

It is possible that Matruchot(1892) may have been right in assuming that Berlese's(1888) species was synonymous with A. oligospora. The original description did not state that the conidiophore was branched or that its nodes were irregularly spaced. Recognition of these features occurred through the work of the preceding men. However, since there is apparently no "type" material it seems best to recognize the species as it has become known as the original A. arthrobotryoides.

The first non-nodular species, A. deflectans Bres., was described by Bresadola(1902). This also represents the first and only description of a species with sessile conidia.

One final item may be included among the early works. Seurat(1920) in a discussion of the work by Maupas (unpublished) stated that Maupas had discovered a species of Arthrobotrys that he called A. strangulans Maupas because it captured nematodes with a collar and killed them by strangulation. He had isolated it in cultures of Cephalobus rigidus in both Argentina and Algiers. However, Seurat stated that Maupas had not published a description of A. strangulans at that time and none has been discovered since. This discussion by Seurat is the first record of constricting rings in the genus Arthrobotrys.

With the work of Charles Drechsler(1933), a new era of modern taxonomic studies was begun on the genus Arthrobotrys and other genera of predaceous fungi. He(1933a) isolated A. oligospora and three similar species from cultures of nematodes and diseased rootlets. Two of the species differed from A. oligospora

only in conidial characteristics, however, the third was also different in that it formed a single terminal head of conidia born on extremely long sterigmata.

Drechsler(1937) described as new species _A. cladodes_ Drechsler, _A. conoides_ Drechsler, _A. musiformis_ Drechsler and _A. dactyloides_ Drechsler. _A. conoides_ and _A. cladodes_ differed from other species of the genus only in the shape and size of the conidia. _A. musiformis_, however, is the first species described that bears conidia on long, sometimes branched sterigmata. It is also the first and only species described with two-dimensional networks. _A. dactyloides_ was the first valid species described that formed constricting rings.

Drechsler(1941) followed the preceding work with a review article which briefly discussed the history of the genus marking its important events. It also covered the species which he had previously discussed in his preceding papers, however, it included no new work on _Arthrobotrys_.

Three years later, Drechsler(1944a) published another extensive article which included the original description of _A. cladodes_ Drechsler var. _macroides_ Drechsler. He designated this isolate as a new variety rather than as a separate species because of its close similarity to _A. cladodes_. It differed only in conidium size and shape and in the fact that it produced long chains of resting bodies in the mycelium.

Shortly after this, Drechsler(1944b) described another new species of _Arthrobotrys_. This species, _A. entomophaga_ Drechsler, was quite different from all other species in that its conidiophores had regularly inflated nodes. It was also unique in that it was the first and only species of _Arthrobotrys_ described that is predaceous on springtails(Collembola), capturing them by

means of aerial, sticky hyphal knobs. Otherwise, all other cultural characteristics were distinctly similar to the other species of Arthrobotrys.

A new species, A. straminicola Pedoplicko(1944) was the first described from the U.S.S.R. This species was distinguished from all of the others in this genus by its exceptionally tiny conidia.

Dollfus(1946) contributed a fairly complete review of the taxonomy of predaceous fungi and their relationship to nematodes. He gathered together much of the previous work and included a complete list of references. None of the work was original.

At this time, Duddington began to work on predaceous fungi in Great Britain. The first of his articles(1950) was taken from a talk he gave at the Quekett Microscopal Institute and was an outline on the predaceous fungi as a whole. This outline included previously known facts about the Zoopagaceae, the Hyphomycetes, and other predaceous fungi.

During an extensive study of isolating predaceous fungi, Duddington obtained an isolate that he(1951b) described as A. robusta Duddington. Its distinguishing characteristics were its branched non-nodular conidiophores and the size and shape of conidia.

Hughes(1953) made a major contribution to the taxonomy of the fungi when he proposed a system of classifying the Hyphomycetes into sections based on the type of conidiophore and conidium development. In this scheme, he placed Arthrobotrys in his Section II which was characterized by the formation of conidia as blown-out ends at the apex of the conidiophore. Each conidium formed terminally on the conidiophore, and was then pushed to one side by an elongation of the conidiophore from a point just directly next to where it was attached. A new conidium was then formed on the new terminal point of the

conidiophore. Thus each conidium was formed in a terminal position on the conidiophore slightly above the last one to form and the condiophore increased in length with the development of each conidium.

Hughes considered A. superba to be a prime example of interrupted conidial formation where the conidiophore alternated between lengths with conidia that are restricted to nodes and lengths without conidia.

The last new species described from the United States was published by Drechsler(1954). This species, A. anchonia Drechsler, differed from the others in having a combination of a single terminal head, obovoid conidia, and constricting rings.

This report was followed by one in which Meyer(1958) described the species A. stilbacea Meyer from the Belgium Congo. This species was described as having both a moniliaceous and stilbaceous form and was reported to be a plant parasite. This was the first time that an organism with either a stilbaceous form or plant parasitic nature had been described as a member of this genus. It seems likely that either of these characteristics might have been sufficient to place the species in a separate genus.

In that same year, Soprunov(1958) described three new species, A. dolioformis Soprunov, A. kirghizica Soprunov, and A. oviformis Soprunov from the U.S.S.R. These species all had branched nodular conidiophores and differed from each other only in conidium size and shape. These descriptions were part of a taxonomic treatment written on the predaceous fungi.

The most recent taxonomic work has been that of Cooke and Godfrey(1964) consisting of a key to common species of several genera of predaceous fungi based on a review of the literature. It included characteristics from both pure and nematode infested cultures.

Biological and Morphological Studies

Morphological and biolgical studies were actually begun by Woronin in (1870). In a study of the morphology of A. oligospora, he discovered hyphal bails and loops which had compounded into networks. He observed them forming both in the mycelium and from the conidia but he did not offer any explanation of their function. It was not until the classic work of Zopf(1888) when he observed them trapping nematodes that the function of these networks was established. He placed both nematodes and A. oligospora in a Giessler chamber and observed this phenomenon under a microscope.

Drechsler(1933a) also studied the networks of A. oligospora. He concluded that at least the internal surface of the loops was highly adhesive and observed that after a nematode was trapped, its integument was penetrated by a hypha which formed inflated organs inside the victim's body. Later (1937) he discovered that assimilative hyphae formed throughout the victim's body and absorbed the internal organs.

Maupas (in Seurat, 1920) first observed constricting rings in a species of Arthrobotrys. He observed that they were collarlike and killed by strangulation.

The second report of a species of Arthrobotrys forming constricting rings came from Drechsler(1933b). He observed that each loop was composed of a three celled ring on a two celled stalk. The ring cells inflated and killed the nematode by squeezing it to death. Later, he(1933c) observed that these rings formed only in cultures infested with mites or nematodes. Because of this, he concluded that some sort of stimulus was necessary to induce their formation.

Two Frenchmen, Comandon and de Fonbrune(1938) studied the mechanism involved in the trapping organs, especially the constricting ring. They found that they could stimulate the ring to close by stroking the inside of it with a micro-manipulator needle. However, Couch(1937) failed to obtain more than a slight swelling this way. Comandon and de Fonbrune also found that the swelling of the cells which results in closure was accompanied by a rapid increase in the volume of vacuoles in the cells of the ring.

The third type of predaceous organ found in Arthrobotrys was first observed by Drechsler(1944). These were aerial organs composed of a globose, sticky distal cell which was born upright on an oblong proximal cell. They formed at the junction of hyphal networks where several hyphae fused. He observed that springtails were captured by their legs adherring to these sticky knobs.

Woronin(1870) also described chlamydospores in the genus Arthrobotrys. He described those formed by A. oligospora as round, pedicellate, and echinulate.

Drechsler(1937) also studied chlamydospore production in A. oligospora. He observed yellow, cylindrical to ellipsoid chlamydospores with a two-layered wall. The outer wall, he concluded, was an extension of the hyphal wall. It covered the thick inner wall which had a noticeable pit in each end.

Initial work on the use of predaceous fungi to control pathogenic nematodes was done by Lindford and colleagues in the period between 1937 and 1939. Lindford(1938) first made a soil survey of the potential agents of biological control existing in the soil. He found eleven nematode trapping fungi, six endozoic predaceous fungi, one fungal parasite of nematode eggs, one protozoan parasite of nematodes, twenty-four predaceous nematodes, three predaceous tardigrades and six mites. In two early experiments, Lindford(1938a, 1939) ran pot culture experiments in which pineapple plants were grown in sterilized

soil with larvae of Heterodera marioni added and with or without the addition of nematode trapping fungi. Of the six fungi used (two isolates of Arthrobotrys oligospora, A. musiformis, Dactylella ellipsospora, Dactylaria candida and Dactylaria thaumasia) only D. ellipsospora showed any degree of effectiveness in controlling nematodes. Since Lindford(1937) had earlier found that he could increase predaceous activity of the fungi by increasing the number of free-living nematodes present in the soil, he(1938) tried experiments similar to the previous ones, but he added organic material to the soil. This increased the populations of free-living nematodes in the soil. The effectiveness of all of the species of fungi was remarkably increased.

Meanwhile in Great Britain, Goodey(1938) found Arthrobotrys oligospora capturing Anguillulina dipsaci on leaves of Calceolaria integrifolia.

A series of papers were written from 1939 to 1941 by a group of Frenchmen who were working on the control of animal pathogens with predaceous fungi. These included: Deschien's(1939) work on the control of Dityocalus filaria in sheep and goats; Roubaud and Descazeaux's(1939) work on the control of Stronglus and Trichonema; Roubaud and Deschien's(1939) work on the control of Strongyloides fulleborni and Ankylostoma duodenale of chimpanzees and man; Deschien's(1939a) work on the control of Strongyloid larvae from monkeys and oxen; Descazeau's (1939) work on control of larvae of the Trichostrongylidae of oxen and sheep; and a summary of the previous works by Deschiens(1939b). All of these men used A. oligospora as one of the test organisms. Deschiens also published three other pertinent papers. The first (1941) covered the use of A. oligospora in capturing Heterodera marioni. The second (1942) discussed culture techniques for heavy spore production and the third by both Deschiens and Lamy(1943) was again concerned with the use of A. oligospora in controlling Heterodera marioni.

A series of papers followed in the next few years indicating that Arthrobotrys played a part in the control of nematodes in nature. Lambert et al(1949) reported A. superba capturing nematodes associated with mushroom destruction. De Wolfe et al (1954 & 1954a) reported A. oligospora in citrus soils capturing Tylenchus semipenetrans.

In 1956, Duddington published an excellent review covering all important biological work to that time. It includes discussions about eelworms and the damage they do, predaceous fungi and their trapping organs, and the studies undertaken by Lindford, Roubard, Descazeaux, Deschiens, and Lamy. In conclusion, Duddington sums up the problems involved with using fungi to control nematodes.

Duddington(1950) also published a paper which included a section on techniques in which he recommended the use of maizemeal agar for isolating Hyphomycetes and potato-carrot agar for maintaining them in pure culture. He also suggested isolating these organisms by touching a microneedle to sterile agar and then to a head of conidia. The conidia readily adherred to the needle and were then transferred to a petri plate.

He(1951b) followed this paper with a preliminary survey of the habitats of predaceous fungi. He isolated various species from leaf mould, partly decayed plant remains, dung, and living bryophytes. From 135 collections of these materials, he recorded 100 species of predaceous fungi.

The most recent work in this field is being done by Pramer and associates of Rutgers. Pramer and Stroll(1959) demonstrated that a broth from the nematode Neoaplectana glaseri would induce trap formation in A. conoides. The substance responsible for the stimulation was designated "nemin". Winkler, Kuyama, and Pramer(1961) developed an assay procedure for "nemin" purification. They have not yet had complete success with it, thus this substance has not yet

been chemically identified. Feder, Everard, and Wootton (1963) demonstrated that all fungi do not respond uniformly to "nemin". Each species of fungi studied apparently had a different requirement before it was effective. A complete summary of the work by Pramer and his associates has been written by Pramer (1964).

The latest work, by Iffland and Allison (1964) showed that although larvae of Meloidogyne hapla would induce trap formation in A. conoides, neither healthy roots nor roots of Lycopersicon esculentum parasitized by the root-knot nematode would do so.

Little is known about the cytology of Arthrobotrys. Hughes (1958) described a type of nuclear development found in one species in Section II described in his paper, however, this appears to bear little similarity to the situation in Arthrobotrys. Much work is needed here.

METHODS AND MATERIALS

The results reported in this work are based on a study of twenty-one isolates of Arthrobotrys. Eleven of these cultures were obtained from the U. S. Army Natick Laboratories Culture Collection and the Centraalbureau voor Schimmelcultures, Baarn, Holland. The remaining ten isolates were obtained from soil and decaying organic matter by the author. The isolates are listed in table 1.

Table 1.

Isolates of *Arthrobotrys*

Culture No.	Name	Source
8416	*A. conoides*	U.S. Army Natick Laboratory
7857	*A. conoides*	U.S. Army Natick Laboratory
7365	*A. superba*	U.S. Army Natick Laboratory
1688	*A. superba*	U.S. Army Natick Laboratory
8418	*A. robusta*	U.S. Army Natick Laboratory
8415	*A. cladodes*	U.S. Army Natick Laboratory
	A. oligospora	Centraalbureau voor Schimmelcultures
	A. conoides	Centraalbureau voor Schimmelcultures
	A. musiformis	Centraalbureau voor Schimmelcultures
	A. cladodes var. *macroides*	Centraalbureau voor Schimmelcultures
	A. arthrobotryoides	Centraalbureau voor Schimmelcultures
101	*A. arthrobotryoides*	Soil and roots, Hackberry Glen, Riley Co., Kans.
130	*A. arthrobotryoides*	Mud, Spillway Park, Pottawatomie Co., Kans.
103	*A. arthrobotryoides*	Soil and roots, Hackberry Glen, Riley Co., Kans.
102	*A. dactyloides*	Soil and roots, city dump, Manhattan, Riley Co., Kans.
136	*A. dactyloides*	Greenhouse soil, Manhattan, Riley Co., Kans.
105	*A. conoides*	Soil, city dump, Manhattan, Riley Co., Kans.
108	*A. conoides*	Soil, Wildcat Creek, Manhattan, Riley Co., Kans.
122	*A. conoides*	Decayed leaves, Wildcat Creek, Manhattan, Riley Co., Kans.
106	*A. oligospora*	Mushroom bed, Kansas City, Mo.
123	*A. superba*	Decaying wood, Wilcat Creek, Manhattan, Riley Co., Kans.

Isolation of Arthrobotrys

Soil and decaying organic material was collected from such areas as woodlands, prairies, stream banks, swampy bogs, greenhouse soil, and compost from mushroom houses. Approximately 100 grams of material from each site was put into a pint plastic bag and sealed with a rubber band. No attempt was made to determine relative numbers of these fungi present in the various types of locations. Interest here was mainly in obtaining as many different strains of *Arthrobotrys* as possible.

Cultures of *Arthrobotrys* were isolated by placing small amounts of the collected material on plates containing maizemeal agar. The media was made by using 15 grams of Diffco Bacto corn meal agar per liter of water. Each plate contained approximately 25ml of the medium. After the soil was placed in the plates, they were kept at room temperature (75°F) and examined under a dissecting microscope at weekly intervals for a three month period. The first indication of the presence of *Arthrobotrys* was the appearance of trapped nematodes. This was followed closely by the development of conidiophores.

When a species of *Arthrobotrys* began to sporulate, it was immediately transferred to a sterile plate of maizemeal agar. This was done by flaming a microspatula, touching it to the agar in a sterile plate and then in turn touching it to a head of conidia. The conidia readily adherred to the agar surface on the spatula and were thus easily transferred to the petri plate of sterile agar.

Study of Isolates in Pure Culture

Several methods were used to observe isolates in pure culture. The gross colony characteristics were noted by making unaided visual observations

of the plates. Actively growing cultures in petri plates were then examined under both the binocular dissecting microscope and the compound microscope. Characteristics such as nodular development, branching, and the number of conidia per head were noted with this type of examination.

Conidium size and shape was then determined by making temporary mounts in lactophenol-cotton blue. All other measurements and all observations of microcharacteristics such as the number of septa in a conidiophore were done from slide cultures.

Slide cultures were made by the following method. First, a moist chamber was made by placing a 9 cm. filter paper in the bottom half of a petri plate. A ¼" diameter glass rod bent into a "u" shape was then placed on the filter paper to keep the slide culture from coming into direct contact with the filter paper. A glass microscope slide and two 22 mm. square coverslips were laid on the rod and then the petri dish was closed and autoclaved. After these were sterilized, two blocks of agar approximately ½" square and 1/8" thick were cut from plates of maizemeal agar and placed on the microscope slide. The agar blocks were then inoculated with conidia or mycelium and covered with a coverslip. The filter paper was dampened with sterile water, and the slide culture stored in an incubator at 75^{o}F until conidiophores formed.

Some slide cultures were examined under a compound microscope while the cultures were still growing and others were made into semipermanent mounts. The semipermanent mounts were made from both the slide and the coverslip if the culture had grown onto them. First the coverslip was removed and the block of agar discarded. The coverslip was placed gently on a clean slide over a drop of mounting medium. A drop of mounting medium was also placed on

the slide in the place where the agar block had been removed with the mycelial growth surrounding it. A coverslip was gently placed over the drop of mounting medium causing it to spread out through the mycelium. The coverslips were then ringed with Zut. Zut is the tradename of a slide ringing compound manufactured by Bennetts Paint Co. of Salt Lake City, Utah.

The two mounting media best suited for examination of cultures were lactolphenol-cotton blue and Phloxin used with 1% KOH. However, both caused a small amount of shrinkage in the hyphae and the spores. The amount of shrinkage in spores was measured using 25 conidia of each of two species. Care was taken that the material did not dry out because of evaporation of the stain. The maximum shrinkage obtained was 3% of the length and 8.5% of the width in conidia of *A. cladodes* and 10.5% of the length and 21.5% of the width in conidia of *A. superba*. The mean was somewhat less than this. Most of the shrinkage occurred after the conidia had been left in the mounting medium over fifteen minutes. Because of this, all measurements for this paper were taken within ten minutes after placing the conidia in the mounting medium.

Studies of Nematode Infested Cultures

Nematodes for culture studies were obtained from soil which was mixed with water to free the nematodes. The water from this mixture was gently poured through a series of wire mesh sieves to separate the nematodes. The nematodes were washed from the sieves into a small amount of water and transferred with a sterile microneedle through three washes of sterile water. They were then placed on plates of maizemeal agar. Enough bacteria were transferred with them to act as a food supply. In approximately one week, nematode cultures began to flourish. At this time, blocks of agar containing mycelium

and conidiophores were cut from colonies of actively growing Arthrobotrys species and placed in the center of the nematode infested plates. These cultures were then studied in the same manner as pure cultures.

Cytological Studies

Nuclei of Arthrobotrys were stained for observation with an Azure A stain process specific for nucleic acid using a modification of the technique used by Huebschman(1952). The material was prepared for staining by two methods. In one, the material was picked from a plate with a needle and affixed to a cover slip with egg albumen. In the other method, isolates were grown in slide cultures and the cover slips which contained mycelium naturally adherring to them were stained without the use of an adhesive. Apparently there is enough exudate from the mycelium to keep it adherred to the cover slip. The staining technique is outlined in table 2.

Table 2.

Azure A Nuclear Staining Process

Solution	Time	Temperature
3 parts alcohol; 1 part glacial acetic acid	30 min.	not critical
Distilled water	dip	not critical
Distilled water	dip	not critical
1N HCl	4-5 min.	60°C.
Distilled water	dip	not critical
Distilled water	dip	not critical
3 drops thionyl chloride in 1% Azure A in water	60 min.	not critical
Distilled water	dip	not critical

After staining, permanent mounts were made by carrying the coverslips through a series of 30%, 50%, 70%, 95% and absolute alcohol at ten minute

intervals. Then they were placed in a mixture of 50% absolute alcohol and 50% xylene for ten minutes and finally into xylene. All coverslips were mounted on slides with Permount.

Photomicrographic Studies

Photomicrographic work was done using an A.O. Spencer Microstar Series 4 microscope with a 35 mm film holder and a Leitz Wetzlar microscope with a 4 x 5 in. camera and Polaroid film holder. Adox KB-14 and Kodax Panotomic X black and white film was used in the 35mm camera. Printing was done on Kodabromide F-4 and F-5 paper. Enlargments were made on an Omega enlarger to the specific magnifications listed for each picture. The photomicrographs were taken under low, high, and oil immersion objectives. All processing was done by the author.

RESULTS

The results are divided into two sections, a general study of the genus *Arthrobotrys* and a taxonomic treatment of its species.

PART I: THE GENUS *ARTHROBOTRYS* CORDA

The following results are based on a study of twenty-one isolates of eight species of *Arthrobotrys*. These were studied in both pure and nematode infested culture.

Colony Characteristics

Most species of *Arthrobotrys* are found in soil containing decaying organic material and take from one week to four weeks to appear in culture after

such material is placed on a petri plate of maizemeal agar. When colonies appear on nematode infested plates, they are composed almost entirely, if not completely of prostrate mycelium running on or below the surface of the substratum Once these colonies are actively growing and sporulating, they may be subcultured into either pure or nematode infested cultures and will sporulate in two days to three weeks.

In pure culture, the colonies are white to pale rose or yellow in color. The mycelium spreads rapidly in a circular or an irregular circular pattern. Colonies are generally composed of both aerial and prostrate mycelium which bear conidiophores.

Four general colony types are found in pure culture. The first of these, exemplified by the species _A. cladodes_ var _macroides_, _A. musiformis_, _A. robusta_ and _A. superba_ is "cushionlike" and composed largely of aerial mycelium. The mycelium intertwines and anastomoses to produce a high, rounded, slow spreading colony. The second major type, found in species _A. superba_ and _A. conoides_, produces prostrate mycelium in the center of a colony and both prostrate and aerial mycelium around the perimeter. The third type of colony exemplified by the species _A. arthrobotryoides_, _A. oligospora_, _A. conoides_, and _A. superba_ is composed of prostrate mycelium which gives rise at intervals to low lying aerial mycelium. This produces a "wool-like" effect. The final colony type, found in _A. conoides_, _A. dactyloides_, and _A. cladodes_ consists entirely of prostrate mycelium. This type of colony in pure culture appears to be rare. Each species may have more than one colony type. Although certain colony types appear more frequently in specific species than others, this does not seem to indicate a relationship between the two.

Mycelium

The mycelium comprising the colonies is septate and contains hyphae 2-7 u wide that frequently fuses (Figs. 3, 4) with itself. The hyphae grow at a rate of approximately 2-15 mm per day. Septa are generally formed 50-100 u back from the tip of the growing hyphae. The length of hypha from the last septum to the tip contains highly concentrated protoplasm. Most of the 10-20 nuclei present in the terminal cell of a hypha are found within 20 u of the tip. Each hyphal cell contains one or more nuclei when the septum forms and from three to ten at maturity.

The mycelium becomes highly vacuolated within two to three days after it forms. These vacuoles differ from the small ones in the newly formed hypha by completely filling the older hyphae.

Predaceous Organs

In nematode infested cultures and sometimes in pure culture, the mycelium forms predaceous organs by modified hyphal branching. The species *A. conoides*, *A. dactyloides*, and *A. oligospora* have formed predaceous organs in pure culture.

The predaceous organs are of three general types, the network, the constricting ring, and the aerial sticky knob. There are two types of networks, the three-dimensional and the two-dimensional. They are both composed of hyphae fused in the form of loops, however, the three-dimensional network contains many 2-5 celled loops which may fuse in any direction. The 3-D network, which is partly prostrate on the substratum and partly raised above it, is extremely efficient as it captures small nematodes by adhesion and larger ones by both adhesion and entanglement.

The two-dimensional network is composed of various numbers of 4-celled

Fig. 1. Solitary chlamydospore x 1240.

Fig. 2. Two chlamydospores in a chain x 1240.

Fig. 3. Hyphal fusion x 1280.

Fig. 4. Hyphal fusion x 1280.

Figure 1

Figure 2

Figure 3

Figure 4

adhesive horseshoe-shaped loops. They appear to fuse in one direction so that the loops of the networks lie in one plane only. They usually rise perpendicular to the substratum. Often the loops do not fuse into a network but remain as a group of single non-constricting rings. These networks are apparently not as efficient as the three-dimensional networks and apparently capture few nematodes. Small nematodes can be held by adhesion, but the larger ones have to wedge themselves into a loop before they are held.

The constricting ring is composed of two parts, a ring and a stalk. The ring is made of three arcuate cells. The first and third cells are fused to the curved stalk which holds the ring perpendicular to the substratum. The stalk contains a short basal cell and a slightly longer distal cell. Several rings form on a single hypha and may be in or on the substratum. As a nematode attempts to crawl through a ring, it stimulates the three ring cells to inflate and thus hold the nematode fast. When one is stimulated to close, all on the same hypha may close. These rings do not appear particularly efficient in capturing nematodes. They are limited by their own diameter to smaller nematodes.

The aerial predaceous organs were not studied due to lack of material. They are known to occur only in the species A. entomopaga.

Once a nematode or springtail is captured by any of the various trapping devices, the predaceous organ sends out a hypha which penetrates the victims integument. After the hypha has passed through the integument, it forms a large round structure which in turn sends out assimilative hyphae which absorb the contents of the nematode, eventually leaving only its integument.

There are five species described as members of this genus that are reported as not having any predaceous organs. They are A. recta, A. rosea, A. straminicola, A. stilbacea, and A. longispora. None of these were obtainable for study at this time.

Conidiophores

The conidiophores in this genus are all hyaline, septate, erect, single, free, and distinct. They range from 20-500 u in length and from 3.5-9 u in width at the base tapering gradually toward the tip. They may originate from either prostrate or aerial mycelium and may be branched or unbranched. Of the eleven common species, four are weakly branched and three strongly branched.

All species have terminal capitate heads of conidia. However, it has been found that in seven species, the conidiophore can elongate to form 3 to 30 additional heads of conidia. In two other species, the conidiophore may form partial nodes or elongate one or two times to form a very weak nodular development.

The spore bearing portion of the conidiophore may be swollen and bear short wartlike sterigmata in a whorled pattern or it may be nonswollen and hold longer sterigmata in a branch-like arrangment.

Conidiophore Development and Spore Formation

The conidiophores arise from the vegetative mycelium, differing from it only in their lesser branching, erect development and production of conidia. After the conidiophore begins to develop, it elongates to the height of the first head before producing any conidia. First, the thin primary wall of the conidiophore tip blows out to form a bud cell (Fig. 5) which is soon set off from the conidiophore by a slight constriction (Fig. 6) that gradually becomes more pronounced. Several nuclei from the multinucleate conidiophore tip may enter the bud cell with the influx of cytoplasm (Fig. 15). In a short time a septum forms across the constriction dividing the conidium from the conidiophore (Fig. 7). After the conidium reaches full size, a horizontal septum

divides it into a larger apical cell and a smaller proximal cell. The nuclei in the conidia divide mitotically and average 12.5 in the proximal cell and 17.3 in the distal cell (Fig. 16).

As the first conidium approaches maturity, the conidiophore elongates slightly from a point just below the level where the first conidium was formed (Fig. 8). The slight elongation of the conidiophore becomes the sterigma upon which a second conidium is formed in a manner similar to the first (Fig. 9). In this way, up to 30 conidia may be formed in a single head (Fig. 10, 11, & 12). Each conidium always forms on an elongation of the conidiophore slightly below the level of the previous one. Because a separate sterigma forms for each conidium, the conidiophore tip may enlarge as each conidium is formed. The nuclei in the conidiophore tip also divide mitotically as conidia form. The mature conidiophore tip bearing a full head of conidia may contain as high as 25 nuclei.

After a head of 5 to 30 conidia is formed, the conidiophore may elongate from just below the last formed conidium and grow several microns before forming a second head in a similar manner (Fig. 13). Such elongations may occur numerous times producing 3 to 30 heads on a conidiophore.

Branches of the conidiophore, when formed, arise some distance below the tip (Fig. 14) rather than immediately below the last formed conidium as in the case of normal conidiophore elongation. Branches may produce conidial heads in the same fashion as the main conidiophores.

Conidia

The conidia are hyaline and uniseptate in all species and have a broad distal end and an apiculate proximal end. Each conidium is borne singly on a

Figs. 5-14. Conidia and conidiophore development.

Fig. 5. Conidiophore tip beginning to swell x 1280.

Fig. 6. Constriction forming between budding conidium and conidiophore tip x 1240.

Fig. 7. First conidium produced; septum forming between conidium and conidiophore x 1280.

Fig. 8. Conidiophore elongation below first conidium x 1280.

Figure 5

Figure 6

Figure 7

Figure 8

Fig. 9. Formation of second conidium x 1280.

Fig. 10. Formation of third conidium x 1280.

Fig. 11. Conidiophore elongation before formation of fourth conidium x 1280.

Fig. 12. Formation of fourth conidium x 1280.

Figure 9

Figure 10

Figure 11

Figure 12

Fig. 13. Conidiophore elongation before formation of a second node x540.

Fig. 14. Conidiophore branch x 1280.

Fig. 15. Newly forming conidium containing 2 nuclei x 1600.

Fig. 16. Conidium showing nuclei x 1536.

Figure 13

Figure 14

Figure 15

Figure 16

sterigma. The shape and size of the conidia vary immensely. They may be ovoid, elongate ovoid, obovoid, elongate obovoid, or ellipsoid. Some are slightly curved while others are preceptibly constricted at the septum. In size, they range from 10 to 50 u long and from 4.5 to 15 u wide. Cells which comprise the conidia may be equal in length or the distal cell may be longer and/or wider than the proximal cell. The distal cell may reach 3X the length of the proximal cell.

Chlamydospores

Chlamydospores (Figs. 1 & 2) are found in many of the species. They are yellow in color, and round to oblong in shape. They are found singly or in chains and may be either intercalary in prostrate hyphae or terminal at the ends of prostrate hyphal branches. They appear only in old cultures.

PART II: TAXONOMIC TREATMENT

Arthrobotrys Corda, Pract-Flora Europaeischer Schimmelbildungen. 1839.

A translation from Latin of the original description of Corda is as follows:

> "Conidiophores erect, simple, septate, nodular; nodes swollen and knotty, knots spirally positioned; spores solitary, sustained on swollen nodes. Spores float on warts, acrogenae, didymae, older spores bicellular, base opening apiculiform, and inside, a solitary, simple, firmly implanted nucleus."

The two keys which follow can be used for the identification of all common species of Arthrobotrys. They exclude species of doubtful validity but they include all species presently reported from the United States.

Characteristics used in the first key are based on a study of isolates grown in pure culture on maizemeal agar incubated at 75° F with the exception

of *A. anchonia*, *A. dolioformis*, *A. entomopaga* and *A. oviformis* which were not obtained in culture. The characteristics of these species were taken from the literature.

These factors should be considered when using the key since certain other culture media such as yeast extract agar and the presence of nematodes will cause variations in some of the characteristics used in the key.

Characteristics used in the second key are based on a study of isolates grown in nematode infested culture on maizemeal agar and/or a review of the literature for the same species as listed above and also *A. robusta*. This key should be used only as a preliminary attempt at classification. Characteristics vary much more widely on nematode infested culture than in pure culture so all precise identification should be done from pure culture. Also, some characteristics such as branching of the conidiophores may not be evident in nematode infested cultures.

Descriptions and illustrations of these species follow the keys in alphabetical order.

Key to Species in Pure Culture

1. Conidia produced in several whorls at swollen nodes along conidiophore--2

1. Conidia produced at the apex of the conidiophore and its branches; occasionally weak nodal development occurs---8

    2. Conidiophores branched--3

    2. Conidiophores unbranched--5

3. Cells of conidia equal in length; septum nonconstricting--*A. arthrobotryoides*

3. Distal cell of conidia distinctly longer than proximal cell; septum constricting------------------------------4

4. Conidia obovoid, distal cell swollen---------------<u>A.</u> <u>oviformis</u>

4. Conidia elongate obovoid, distal cell not swollen------------------------------------<u>A.</u> <u>dolioformis</u>

5. Cells of conidia equal in length; septum nonconstricting--6

5. Distal cell of conidia distinctly longer than proximal; septum constricting--7

6. Conidiophore head irregularly swollen; conidia on wartlike sterigmata in a tight capitate head-----------------------------------<u>A.</u> <u>superba</u>

6. Conidiophore head regularly inflated; conidia on long sterigmata in loose capitate heads---------------------------------------<u>A.</u> <u>entomophaga</u>

7. Conidia obovoid to pyriform; distal cell length 2X proximal--<u>A.</u> <u>oligospora</u>

7. Conidia elongate obovoid; distal cell length $1\frac{1}{2}$X proximal--<u>A.</u> <u>conoides</u>

8. Conidiophores unbranched--9

8. Conidiophores branched--11

9. Cells of conidia equal in length--------------------------<u>A.</u> <u>dactyloides</u>

9. Distal cell of conidia distinctly longer than proximal cell--10

10. Conidia obovoid; on short wartlike sterigmata--<u>A.</u> <u>anchonia</u>

10. Conidia elongate ellipsoid, slightly curved; on long sterigmata-------------------------<u>A.</u> <u>musiformis</u>

11. Conidiophore tip nonswollen; conidia large (20x40u); born on long sterigmata------------------------------<u>A.</u> <u>robusta</u>

11. Conidiophore tip swollen; conidia small (11x 22u); born on wartlike sterigmata-----------------------------<u>A.</u> <u>cladodes</u>

Key to Species in Nematode Infested Cultures

1. Predaceous organs consisting of sticky
knobs--*A. entomopaga*

1. Predaceous organs of networks or
constricting rings--2

    2. Constricting rings---3

    2. Predaceous networks--4

3. Conidia obovoid, distal cell of conidia
longer than proximal cell-------------------------------------*A. anchonia*

3. Conidia elongate ellipsoid, cells of
conidia equal in length------------------------------------*A. dactyloides*

    4. Conidiophore tip swollen, and
    forming conpact head of conidia-------------------------------------5

    4. Conidiophore tip nonswollen, often
    forming loose head of conidia--------------------------------------11

5. Conidia constricted at the septum--------------------------------------6

5. Conidia not constricted at the septum----------------------------------9

    6. Conidia obovoid to pyriform---7

    6. Conidia obconical to elongate obovoid------------------------------8

7. Conidia 16-29(24)u long, 8-5(13.6)u wide with proximal
cell 4.2-11.2(8.7)u; usually nodular; groups of
conidiophores form in clumps---------------------------------*A. oligospora*

7. Conidia 21-40(30)u long, 7-12(9)u wide with
proximal cell 9.8-24(17)u long; may be
nodular or branched---*A. oviformis*

    8. Distal cell of conidia distinctly
    swollen, may be nodular-------------------------------------*A. coniodes*

    8. Distal cell of conidia no dis-
    tinctly swollen, may be nodular
    or branched---*A. dolioformis*

9. Conidia elongate ovoid--10

9. Conidia ovoid to obovoid, 12.6-32.2(20.7)u long and 4.2-12.6(8.7)u wide------------------------A. arthrobotryoides

10. Distal cell of conidia slightly tapered; conidia 10-18u long, 5-9u wide; conidiophore non-nodular--------------------------------A. cladodes

10. Distal cell of conidia not tapered; conidia 16.8-26.5u long, 5.6-11.2u wide; conidiophore may have 2 nodes-------------------A. superba

11. Conidia formed in a loose head on long sterigmata; conidiophore simple----------------------------A. musiformis

11. Conidia formed in a compact head on shorter sterigmata; conidiophores branched-------------------A. robusta

1. Arthrobotrys anchonia Drechsler. Mycologia 46:6, 762-763. 1954.

A translation from Latin of the original description by Drechsler is as follows:

"Mycelium on nematode-infested substrata usually scanty, spreading; vegetative hyphae colorless, septate at moderate intervals, mostly 2u to 5u wide, often (especially in presence of nematodes) giving rise on straight or curving stalks to circular rings in perpendicular positions; the stalks 7 to 25u (commonly 7 to 15u) long, 4 to 6u wide, usually composed of 2 unequal cells, the proximal cell 2 to 8u (mostly 2.5 to 5u) long, the distal cell 5 to 17u (mostly 5 to 12u) long; the rings, measuring 20 to 42u in diameter and surrounding a circular or rounded triangular aperture 12 to 32u wide, being regularly composed of 3 arcuate segments 15 to 35u long, 2.8 to 4.7u wide at the ends and 4 to 7.5u wide in the middle--the first and third segments being united to one another as well as to the end of the stalk; on entrance of a nematode into the aperture the arcuate cells contracting abruptly, all three indenting the animal broadly and deeply, thereby strongly constricting it and soon disabling it, then perforating its integument and extending through its body assimilative hyphae to appropriate its fleshy contents; the assimilative hyphae becoming septate at moderate intervals, for the most part 2.5 to 4.2u wide but often terminating in one or more bulbous enlargements or swollen cells 5 to 8u wide. Conidiophores colorless, erect, in later stages containing 3 to 8 cross-walls, 350 to 500u high, 4 to 6u wide at the base, tapering gradually upward to a width of 2.5 to 3.5u, at the tip often furnished with several short stout spurs on which are borne collectively 3 to 8 conidia in a handsome head; conidia colorless, elongate obovoid, mostly 29 to 43u (average 35u) long, 15 to 19u (average 16.8u) wide, rarely biseptate, usually uniseptate with the proximal cell commonly 8 to 18u (average 23.5u) long.

Capturing and consuming nematodes of different species, it occurs in decaying plant residues near Laplace, Louisiana, and also in decaying

stems and roots of Phaseolus vulgaris L. near Fort Lauderdale, Florida."

A. anchonia (Fig. 17) appears most closely related to A. dactyloides. These species are unique in forming three celled constricting rings in nematode infested cultures. Both have unbranched non-nodular conidiophores with nonswollen tips and each bears a small number of conidia on sterigmata. However, A. anchonia has elongate obovoid conidia with unequal cells while A. dactyloides has ellipsoid conidia with equal cells.

A. anchonia has been reported only by Drechsler from his original collection and no cultures were available at this time for study.

2. Arthrobotrys arthrobotryoides (Berlese) Lindau, in Rabenhorst's Kryptogamen-Flora. pp. 371. 1907.

Syn: Cephalothecium roseum Corda var. arthrobotryoides Berlese, Malpighia 1:245-246. 1888.
Didymozoophaga arthrobotryoides Soprunov and Galiulina, Microbiology (in Russian) 20:489-499. 1951.
Arthrobotrys superba Corda var irregularis Matr, Recherches sur le Development de Quelques Mucedinees. 1892.

A translation from Latin of the original description by Berlese is as follows:

"Colony dense, rose-colored, spreading; vegetative hyphae non-erect, hyaline, winding, branched, septate; conidiophores erect, varying in length, straight, tapering upward from base, swollen tip with sterigmata, immediately (rarely) adding nodes with sterigmata toward the middle or near apex, 150-200u long, 5-7u wide, pale rose; conidia ovoid elongate, similar to Trichothecium roseum, uniseptate, top rounded, base in apiculum, frequently blunt ended, slightly constricted at septum, anucleate, distal cell slightly swollen, born radially on sterigmata, pale rose, 20-22u X 9-10u."

The colony in pure culture is white or pale rose, spreads rapidly and sometimes shows a concentric circular pattern of sporulation. The mycelium is mostly prostrate, hyaline, septate, highly branched, 2.5-5.5u wide and

Fig. 17. A. anchonia. Reproduced from Drechsler(1954).

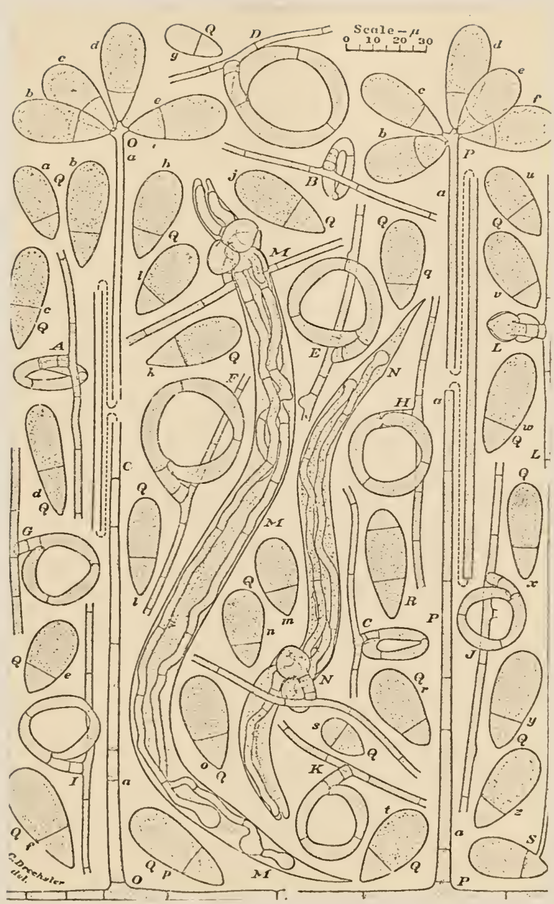

Figure 17

often forms hyphal fusions. Aerial mycelium, which appears on plates as small "wool-like" tufts often fuses longitudally into coarse strands above the substratum before bearing conidiophores. The conidiophores, which form in less than a week, are highly branched (Figs. 22-25), 4-5.5u wide at the base, and taper to 1.5-3u wide below the swollen tip. They reach a length of 300-400u tall at the first node when originating from prostrate mycelium, 10-200u at the first node when originating from aerial mycelium, and often elongate to form 2-5 more nodes of conidia (Fig. 26) at irregular distances. The conidiophore tip is irregularly swollen (Fig. 19) and bears up to 25 conidia in a single capitate head. Conidia (Fig. 18) are solitary on blunt sterigmata (Figs. 20 & 21), two-celled, and ovoid to obovoid. The distal cell of the conidium is slightly swollen, and the septum is occasionally constricting. The conidia have a blunt, rounded distal end, an apiculate base and are 12.6-32.2 (20.7)u long, 4.2-12.6 (9.5)u wide. Chlamydospores are unknown.

Colonies in nematode infested culture are indistinct. The mycelium is prostrate, septate, hyaline, branched and 3-6u wide. It forms adhesive loops which are often compounded into networks. The conidiophores are unbranched, 300-450u in length, 5-7u wide at the base and taper gradually to 4-5u below the apex. They usually bear a single terminal capitate head of 5-15 conidia but they sometimes elongate to form two additional heads. The conidia are the same as in pure culture.

This organism was first described by Berlese in 1888 as *Cephatothecium roseum* Corda var *arthrobotryoides* Berlese. This was probably due to a misinterpretation of conidiophore characteristics. After Matruchot redefined conidial formation in *Cephalothecium* and *Arthrobotrys*, he transferred this variety to the genus *Arthrobotrys* as a synonym of *A. oligospora*. This was

Fig. 18. Conidia x 1280.

Fig. 19. Conidiophore tip x 1280.

Figs. 20-21. Sterigmata x 1280.

Figure 18

Figure 19

Figure 20

Figure 21

Figs. 22-25. Conidiophore branching.

Fig. 22. Rachis-type branching x 540.

Fig. 23. Combination of rachis-type and side branching x 540.

Fig. 24. Typical branching habit x 125.

Fig. 25. Side branch x 540.

Figure 22

Figure 23

Figure 24

Figure 25

Fig. 26. Nodular development of conidiophore x 1280.

Figure 26

apparently done from the description since he gave no reference to any actual culture study. However, his writings indicate that he may in fact have handled material of A. arthrobotryoides as we know it today without realizing it. His account and illustrations of A. superba var. irregularis has many features in common with A. arthrobotryoides (Berl.) Lindau and has thus been considered to be synonymous with it.

Lindau (1907) raised A. arthrobotryoides to specific rank. He did not add any new material to the previous description when he did this. In fact, he left out of his description the information that the distal cell was slightly larger than the proximal cell.

Drechsler (1944) found that the conidiophore had a limited nodular development. He also noted that the conidia were not always in tight heads but that each conidium was often born on a separate short branch giving the conidiophore a rachis like appearance.

This species appears to be most closely related in morphology to two species described by Soprunov, A. doliformis and A. oviformis. All three have branched and nodular conidiophores, but their conidia vary in size and shape. A fourth species by Soprunov, A. kirghizica which is discussed in the next section may also be synonymous with A. arthrobotryoides.

Material Studied: Unnumbered culture from Centraalbureau voor Schimmel cultures, Baarn, Holland; No. 1 and 3 from soil and roots, Hackberry Glen, Riley Co., Kansas, Karen Haard, February, 1963; No. 30 from mud, Spillway Park, Pottawatomie Co., Kansas, Karen Haard, August, 1964.

3. Arthrobotrys cladodes Drechsler, Mycologia 29:4, 459-464. 1937.

Syn: Trichothecium cladodes Soprunov Predaceous fungi - Hyphomycetes and their application in the control of pathogenic nematodes (in Russian). 1958.

A translation from Latin of the original description by Drechsler is as follows:

> "Mycelium spreading; vegetative hyphae hyaline, septate, except for occasional storage filaments that are densely filled with protoplasm and up to 11u wide measuring mostly 2 to 7u in diameter, often especially in the presence of nematodes giving rise to hyphal bails and loops, which, though at first discrete, are later frequently compounded into more or less extensive networks; the bails and networks capturing nematodes through adhesion and entanglement, perforating the integument of each animal and intruding one or more globose mortiferous excrescences from which are extended assimilative hyphae to appropriate the fleshy contents. Conidiophores hyaline, erect, septate, frequently more or less branched, 200 to 400u high, 4 to 7u wide at the base, tapering gradually upward to a width of 2.5 to 4u below the irregularly expanded, globose or somewhat coralloid tip whereon are borne 5 to 30 conidia in usually dense capitate arrangement. Conidia hyaline, ellipsoid or elongate obovoid, mostly 11 to 18u (average 14.7u) long, 6.2 to 8.8u (average 7.3u) wide, uniseptate, the upper cell often approximately of the same size as the lower or slightly larger, but occasionally somewhat smaller. Chlamydospores not known.
>
> Capturing and consuming nematodes commonly measuring up to .5 mm. in length and mostly referable to the genera Acrobeles."

A study of one culture of Arthrobotrys cladodes verifies most of the characteristics given in Drechsler's description. In pure culture, this species produces radially spreading prostrate mycelium which is hyaline, branched, septate, 2-6u wide, and often forms hyphal fusions. Conidiophores appear approximately twenty days after inoculation onto maizemeal agar. They are erect, septate, very delicate, 150-350u in length, 3-5u wide at the base and they taper to 1.2-3u wide just below the irregularly swollen tip. These conidiophores are highly branched and after forming an initial head of conidia, they may elongate to form one or two additional heads. The conidiophore tips are irregularly swollen and produce 20-30 conidia, each on a wartlike sterigmata, in a tight capitate head. The conidia are 10-18 (15)u long, 5-9 (7.5)u wide and contain two cells that are equal in length. The conidia are rounded at both the proximal and the distal ends. However, they do have a sharp point at the base where they attach to the conidiophore.

Nematode infested cultures contain only prostrate mycelium which forms loops that become anastomosed into adhesive networks. The conidiophores are fewer and more scattered than in pure culture colonies. They are also larger, unbranched, and non-nodular. They form 10-15 conidia on sterigmata on irregularly swollen heads. The conidia are the same size and shape as those formed in pure culture. Chlamydospores are unknown.

Material Studied: No. 8415 from culture collection, U.S. Army Natich Laboratories.

3a. Arthrobotrys cladodes var. macroides Drechsler, Mycologia 36:2, 138-145. 1944.

Syn: Trichothecium cladodes var. macroides Sopr. Predacious fungi-Hyphomycetes and their application in the control of pathogenic nematodes. (in Russian) 1958.

A translation from Latin of the original description by Drechsler is as follows:

"Mycelium spreading; vegetative hyphae hyaline, septate, at first varying in width mostly from 2 to 7u, some later becoming wider and then occasionally attaining a diameter of 11u, in their young condition often, especially in the presence of nematodes, giving rise to sturdy hyphal bails and loops, which, though discrete in the beginning, are later frequently compounded into more or less extensive networks; the bails and networks capturing nematodes through adhesion and enmeshment, then perforating the integument of each captured animal and intruding one or more globose mortiferous excrescences from which are extended assimilative hyphae to appropriate the fleshy contents. Conidiophores hyaline, erect, septate, simple or somewhat branched, mostly 75 to 300u high, 2 to 6u wide at the base, 1.5 to 2.5u wide below the tip which frequently is somewhat widened or irregularly lobed and on which are borne 5 to 30 conidia in capitate arrangement. Conidia hyaline, elongate ellipsoidal or elongate obovoid, rounded at the distal end, provided with a distinct apiculum-like basal prominence at the proximal end, 13 to 26u mostly 15 to 21u (average 17.6u) long, 5 to 8.2u (average 6.4u) wide, divided by a cross-wall at the middle, above the middle, or below the middle, the upper cell 5.5 to 13.4u (average 8.4u) long, the lower cell 6.4 to 12.4u (average 9.2u) long. Resting bodies formed tardily in the substratum, faintly yellowish, filled with pronouncedly globuliferous contents, mostly intercalary, simple or somewhat branched, commonly 50 to 250u long, usually composed to 5 to 20 segments measuring individually 7u to 35u in length and 7u to 20u in width."

Three isolates of this have been studied and each fits very closely the description given by Drechsler. In pure culture, all three colonies are irregularly shaped and cushionlike. Thick white aerial mycelium rises high above the substratum and intertwines and readily fuses. The mycelium is 1-6u wide, hyaline, septate and branched. Erect hyaline conidiophores, 75-300u in length form in two to three weeks. They are 3.5-7u wide at the base, taper to a width of 2-3u just below the swollen tip (Fig. 28) and contain 3-7 crosswalls. Most of them are highly branched and a few elongate to form 1-3 additional heads of conidia (Fig. 29). Each conidiophore holds 5-20 conidia on wartlike sterigmata in a tight capitate head. These conidia (Figs. 27, 30) are hyaline, uniseptate, elongate obovoid to oblong ovoid, 14-35 (20)u long, and 5.5-11.5 (7)u wide. The distal end is broadly rounded, the proximal end is strongly pedicellate and the two cells are nearly equal in length.

Mycelium in nematode infested cultures is prostrate, septate and hyaline. The colony produces a profusion of erect, non-nodular, unbranched conidiophores slightly larger than those in pure culture. Each conidiophore bears 5-15 conidia like those in pure culture on sterigmata on its swollen tip. This culture also forms adhesive loops which are often compounded into networks.

Both varieties of _A. cladodes_ differ from other species in having a combination of branched conidiophores, limited nodular development, and very small conidia. They differ from each other in conidia size and shape. _A. cladodes_ var. _macroides_ had larger, strongly pedicellate conidia while _A. cladodes_ var. _cladodes_ has conidia which average 5u shorter and which have a brief pointed apex. _A. cladodes_ var. _macroides_ is also reported to have resting bodies but this was not observed in the material used in this study.

Fig. 27. Conidia x 1240.

Fig. 28. Conidiophore tip x 1240.

Fig. 29. Nodular development and branching of conidiophore x 125.

Fig. 30. Conidium x 1240.

Figure 27

Figure 28

Figure 29

Figure 30

Material Studied: Unnumbered cultures from Centraalbureau voor Schimmelcultures (one identified as A. conoides); No. 8416 from culture collection, U. S. Army Natick Laboratories.

4. Arthrobotrys conoides Drechsler, Mycologia 29:4, 473-477. 1937

A translation from Latin of the original description by Drechsler is as follows:

> "Mycelium spreading; the vegetative hyphae hyaline, septate, except for occasional storage filaments that are densely filled with protoplasm and up to 12u wide, measuring mostly 2u to 8u in diameter, often, especially in the presence of nematodes, giving rise to hyphal bails and loops, which though at first discrete are later frequently compounded into more or less extensive networks; the bails and networks capturing nematodes through adhesion and entaglement, perforating the integument of each animal and intruding one or more globose mortiferous excrescences from which are extended assimilative hyphae to appropriate the fleshy contents. Conidiophores hyaline, erect, septate, usually not branched, mostly 4 to 8u wide at the base, tapering gradually to a width of 2.5 to 5u in attaining a height of 150 to 400u before bearing on a globose or more irregularly expanded tip as many as 30 conidia in dense capitate arrangement; subsequently often, following repeated elongation, giving rise successively to additional clusters of conidia. Conidia hyaline, obconical, somewhat flattened at the base, broadly rounded at the tip, usually perceptibly constricted at the septum, 19 to 42u (average 30u) long, 8 to 15u (average 12u) wide, the lower cell measuring 8 to 17u (average 12.5u) in length. Chlamydospores yellowish, globose or prolate ellipsoidal, 18 to 25u in diameter, or sometimes narrower, oblong-cylindrical, 30 to 50u long and approximately 15u wide.
>
> Capturing and consuming nematodes measuring up to .6 mm. in length, referable to the genera Acrobeles, Acrobeloides, Cephalobus, Diplogaster, Diploscapter, Plectus and Rhabditis, it occurs in decaying plant remains and in leaf molds, often outdoors but especially abundantly in greenhouses, near Beltsville, Md., and in Arlington, Va."

A study of four isolates in pure culture verifies the following characteristics of A. conoides. The colonies differ in appearances. Two isolates (No. 7857 and No. 108) when grown on maizemeal agar produce prostrate mycelium in the center of the colony, but have a circle of white aerial and prostrate mycelium around the periphery. Another isolate (No. 105) forms only prostrate

Fig. 31. Conidia x 1040.

Fig. 32. Pure culture network x 1240.

Fig. 33. Nodular development of conidiophores x 540.

Fig. 34. Conidia x 1240.

Figure 31

Figure 32

Figure 33

Figure 34

the conidia average 32u long, 13u wide and contain a proximal cell 13u long. The chlamydospores are the same as in pure culture.

Arthrobotrys conoides appears most closely related to A. superba and A. oligospora. All three species have unbranched and nodular conidiophores. The main difference between these species is the fact that A. conoides has obconical conidia, A. oligospora has pyriform conidia and A. superba has elongate ovoid conidia.

Material Studied: No. 7857, from Culture Collection, U.S. Army Natick Laboratories; No. 105, from soil, city dump near Manhattan, Riley Co., Kansas, Karen Haard, April 1963, No. 108, from soil, Wildcat Creek near Manhattan, Riley Co., Kansas, Karen Haard, April 1963; No. 122, from decayed leaves, Wildcat Creek near Manhattan, Riley Co., Kansas, Karen Haard, April 1963.

5. Arthrobotrys dolioformis Sopr. Predaceous fungi-Hyphomycetes and their application in the control of pathogenic nematodes (In Russian). 1958.

Syn: Didymozoophaga dolioformis Sopr. Microbiology (In Russian) 20:489-499. 1951

A translation from Latin of the original description by Soprunov is as follows:

"Colony cushion-like, yellow-grey; vegetative hyphae non-erect, branched, septate, hyaline, 4-7u wide, trapping loops around 40u wide, circular or arch-like, frequently forming networks; the adhesive snare capturing nematodes. Conidiophores erect, septate, hyaline, fairly flexible, always branched, 200-400u tall, base 7-8.5u wide, tapering upward; conidia hyaline-rose, oblong-obovoid, uniseptate with septum always constricted, two cells unequal, distal cell 3 times proximal cell. Conidia around 28.4u long (23.5-32.5u), 11.5u wide (9-14.5u). Nematodes caught and consumed. Habitat in decaying plant material, Askhabad, Turkmenia, U.S.S.R."

A. dolioformis (Fig. 35) appears most closely related to A. arthrobotryoides, A. kirghizica and A. oviformis. All four species have nodular and branched conidiophores with swollen tips and all four form trapping networks. A. dolio-

Fig. 35. _A_. _dolioformis_. Reproduced from Soprunov(1958)

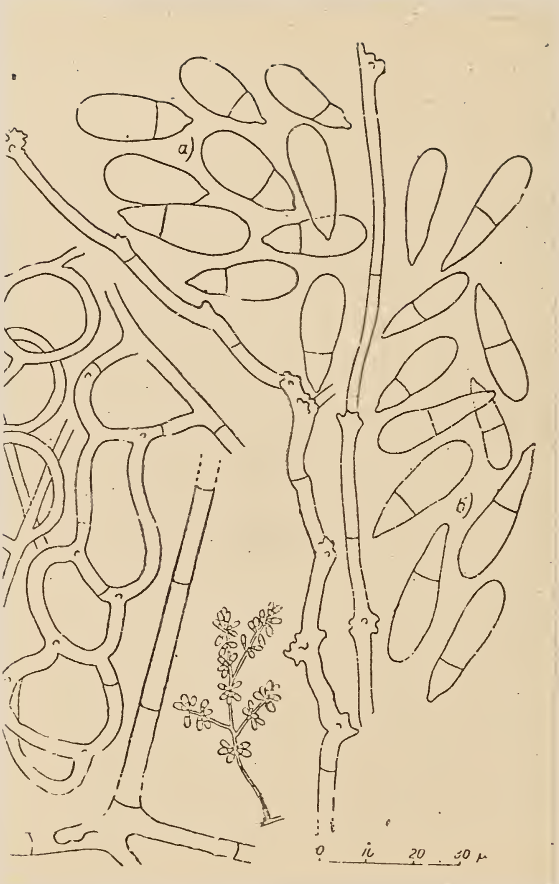

Figure 35

formis differs from the other three by having obovoid-oblong conidia which are constricted at the septa.

No material was available for a study at this time.

6. Arthrobotrys dactyloides Drechsler, Mycologia 29:4, 482-487. 1937.

Syn: Dactylaria dactyloides Sopr., Predaceous fungi-Hyphomycetes and their application in the control of pathogenic nematodes. (In Russian). 1958.

A translation from Latin of the original description by Drechsler is as follows:

"Mycelium spreading; the vegetative hyphae hyaline, septate, mostly 2 to 5u wide, often, especially in the presence of nematodes, producing underneath and at right angles to their respective axies approximately circular rings, 20 to 32u in diameter, composed individually of 3 arcuate cells, 12 to 28u long, 4.5 to 7u wide in the middle and 2.5 to 6u wide at the ends--the first and third of the cells being united to one another as well as to the distal part of a somewhat curved sturdy supporting branch 7 to 14u long, 4 to 5u wide, and consisting usually of 2 cells whereof the proximal one is generally the shorter; following ensnarement of a nematode, the individual ring through contraction and inflation of its component arcuate cells constricting the animal to death or into a state of reduced activity preceding death, then perforating the integument and giving rise to assimilative hyphae that appropriate the fleshy contents. Conidiophores hyaline, septate, erect, mostly 200 to 400u high, 4 to 6u wide at the base, tapering gradually upward to a width of 2.5 to 3.5u at the tip, there bearing on sterigmata 1 to 5u long and 2 to 3u wide, from 4 to 13 conidia usually in a single loose head, more rarely in two somewhat distinct clusters. Conidia hyaline, usually elongate ellipsoidal or somewhat digitiform, straight or slightly curved, tapering noticeably from the broadly rounded wider distal end toward the narrower truncate basal end, 32 to 48u (average 41.6u) long, 7 to 9.5u (average 8.4u) wide, with the single septum 16 to 23u (average 20.5u) from the base; but occasionally becoming wider and shorter, measuring as much as 18u in width and as little as 25u in length, then often 2-septate, with the inflated middle cell greatly exceeding the end cells in size.

Capturing and consuming nematodes measuring up to .6 mm. in length, referable to the genera Acrobeles, Acrobeloides, Cephalobus, Diplogaster, Diploscapter, Plectus, Rhabditis and Mononchus, it occurs in decaying leaves and roots of many plants near Beltsville, Md., and also in leaf mold in deciduous woods in Arlington, Va."

The following account is based on a study of two isolates of A. dactyloides. In pure culture, the colonies are thin, white, and widely scattered. Each colony is composed of highly branched, septate, hyaline mycelium which varies from 2-6u wide. Conidiophores appear on both the aerial and prostrate mycelium within 2½-3 weeks after inoculation. The conidiophores (Fig. 36) are erect, non-nodular, unbranched, 3.5-5.5u wide at the base and taper to 2.2-3.5u wide just below the tip. They reach a height of 200-400u where they form a single terminal loose capitate head. Occasionally a few conidiophores will elongate to produce one or two additional nodes or they will form a single lateral side branch. The nonswollen conidiophore tip (Fig. 36) bears 4 to 10 sterigmata which are 5u long and 1½-2½u wide. Each sterigmata produces a simple slightly curved, uniseptate, elongate ellipsoid conidium (Fig. 37). The conidia measure 32-45 (38)u long and 6-10 (8)u wide. The proximal cell measures 15-25 (20)u long. Chlamydospores are produced and are intercalary, round, single, yellow and up to 15u in diameter. Constricting rings may form in old desiccated cultures. They are composed of three arcuate cells on a two celled stalk.

In nematode infested cultures, the colonies are composed entirely of hyaline, septate, branched, prostrate mycelium. Ring formation (Fig. 38) is similar to that in pure culture but it is much more prevalent than in pure culture. The rings are borne directly on or in the substratum at an angle perpendicular to the surface. Several rings are often found equally spaced on a single hyphae, and often if one ring closes, all rings on the hyphae will close. The conidiophores, conidia and chlamydospores are the same as those described in pure culture.

A. dactyloides appears most closely related to A. anchonia. These two

Fig. 36. Conidiophore x 540.

Fig. 37. Conidium x 1040.

Fig. 38. Nematode in constricting ring x 1600.

Figure 36

Figure 37

Figure 38

species are the only ones in this genus to form constricting rings. Also, they both have non-nodular, unbranched conidiophores. They differ from each other in the size and shape of conidia. A. anchonia has elongate obovoid conidia with unequally sized cells while A. dactyloides has elongate ellipsoid conidia with equal sized cells.

Material Studied: No. 102, from soil and roots, city dump, Manhattan, Riley Co., Kansas, Karen Haard, March 1963; No. 36, from wet greenhouse woil around some Marchantia, Manhattan, Riley Co., Kansas, Karen Haard, October 1965.

7. Arthrobotrys entomophaga Mycologia 36:382-299. 1944.

A translation from Latin of the original description by Drechsler is as follows:

"Mycelium spreading; the ordinary vegetative hyphae long, filamentous, colorless, septate at moderate intervals, mostly 2-3u wide, often creeping on the surface of the substratum and over rather long distances only sparsely branched, but at intervals widening locally and from the widened portions giving off prostrate branches, mostly 3 to 6u wide and spaced 10 to 40u apart, which unite by anastomosis into a network and thereupon give rise to numerous erect aerial predaceous organs; these organs usually uniseptate, the lower cell stalk-like, cylindrical, or tapering upward, mostly 7 to 17u long and 2 to 5u wide, supporting aloft an ovoid or prolate ellipsoidal distal cell usually measuring 8 to 13u in length by 4.5 to 8u in width and soon becoming surrounded by an envelope of adhesive secretion effective in holding any suitable roaming springtail, which then is invaded throughout by branching assimilative hyphae 4 to 8u wide. Conidiophore erect, colorless, meagerly septate, 75 to 175u tall, 3 to 4.5u wide at the base, about 2.5u wide farther upward, often somewhat inflated at the top from which are given off 3 to 8 simple or branched sterigmata, 2 to 7u long, whereon are borne collectively 3 to 10 conidia in loose capitate arrangement; additional conidial clusters often being produced following renewed axial elongation. Conidia colorless, cylindrical or somewhat clavate, 15 to 28u long, 4.5 to 5.5u wide, broadly rounded at the tip, often minutely pedicellate below, uniseptate, the 2 cells not pronouncedly unequal as a rule even though the lower cell is often slightly longer than the upper one.

> Capturing and consuming minute springtails referable to a species of Sminthurides very similar to S. (Sphaeridia) serratus, and occasionally also destroying various nematodes including Plectus parvus, it occurs in decaying roots of Polygonum pennsylvanicum in Arlington, Va."

Arthrobotrys entomopaga (Fig. 39) is the only species of this genus known to form erect, aerial predaceous organs from prostrate networks of hyphae, and to capture springtails (Collembola). When Drechsler described it, he had not yet been able to grow it in pure culture or in any abundance on springtail infested plates. From what he observed, the two-celled conidia which were born on sterigmata on inflated tips of nodular coniophores related it to the other species of this genus.

This is the only known record of A. entomopaga and no material was available for study.

8. Arthrobotrys musiformis Drechsler, Mycologia 29:4, 477-482. 1937.

Syn: Trichothecium musiformis Sopr. Predaceous fungi-Hyphomycetes and their application in the control of pathogenic nematodes. (In Russian). 1958.

A translation from Latin of the original description by Drechsler is as follows:

> "Mycelium spreading; the vegetative hyphae hyaline, septate, mostly 2 to 9u wide, often, especially in the presence of nematodes, giving rise to horseshoe-like hyphal arches and loops that may remain discrete, or in numbers not usually exceeding 6 may be compounded into networks--the individual circular loops mostly composed of 3 to 5 arcuate cells surrounding an aperture 15 to 25u wide; the loops and networks capturing nematodes through adhesion and entanglement, perforating the integument of each animal and intruding one or more globose mortiferous excrescences from which are extended assimilative hyphae to appropriate the fleshy contents. Conidiophores hyaline, septate, erect, not branched below, 200 to 500u high, 5 to 9u wide at the base, tapering upward gradually to a width of 2.5 to 4u near the tip, where are borne on divergent, slightly tapering, simple or branched sterigmata, mostly 2 to 3u wide and 3 to 10u long, usually 5 to 15 conidia in loose capitate arrangement. Conidia hyaline, ellipsoid, straight or slightly curved, broadly rounded at the wider distal end, tapering noticeably toward the slightly protruded base, 22 to 44u (average 33.9u) long, 7.5 to 12.7u (average 10.4u) wide,

Fig. 39. A. entomopaga. Reproduced from Drechsler(1944).

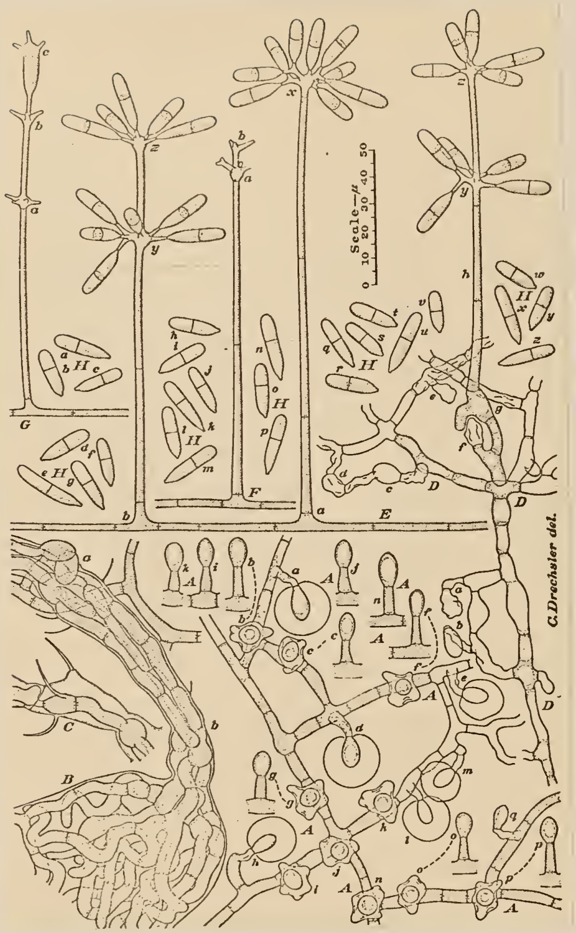

Figure 39

the lower and smaller cell 8 to 16.4u (average 11.7u) long. Chlamydospores yellow, globose or less frequently ellipsoidal, mostly 14 to 22u (average about 17.5u) in diameter.

Capturing and consuming nematodes measuring up to .6 mm. in length, referable to the genera Acrobeles, Acrobeloides, Cephalobus, Diplogaster, Diploscapter, Plectus and Rhabditis, it occurs in decaying spinach roots near Norfolk, Va., in potting soil near Coconut Grove, Fla., and in leaf mold near Beltsville, Md., and in Arlington, Va."

A study of one culture of Arthrobotrys musiformis shows the same characteristics described by Drechsler (1937) and later confirmed by Duddington (1950). The colony in pure culture is white and cushion-like in appearance, and produces both prostrate and aerial mycelium which branches and anastomoses above the substratum to give the cushion-like appearance. The mycelium is hyaline, septate, branched, and 3-5u wide. Both the aerial and prostrate mycelium produce conidiophores which appear in 1-2 weeks, and are non-nodular and unbranched except in rare incidences (Fig. 44 & 45). They are 5.5-7u wide at the base and taper to 2-3u wide just below the level of the head which appears at a height of 200-400u. The conidiophore tip ends in 5-15 short branched sterigmata (Fig. 41, 42) which are 9-21u long and 2-3u wide. Since a single uniseptate conidium forms on each sterigmata, a loose capitate head is produced (Fig. 43). The conidia (Fig. 40) are curved, alongate ellipsoid in shape, and have a broadly rounded distal end and a blunt tapered proximal end. They are 28-49 (35)u long, 8-16 (10.8)u wide, with proximal cells 8-18 (11.5)u long. The ratio of the distal cell to the proximal is three to one. In this isolate, networks did form in numbers of 5-10 per plate in old cultures (Fig. 41, 46).

In nematode infested cultures, the colony is composed entirely of prostrate mycelium. It bears simple horseshoe-like arches in profusion which are usually composed of 4 cells. Although they are generally produced singly, sometimes

Fig. 40. Conidia x 1240.

Fig. 41. Branched sterigmata on conidiophore tip x 540.

Fig. 42. Conidiophore tip x 540.

Fig. 43. Head of conidia x 540.

Figure 40

Figure 41

Figure 42

Figure 43

Figs. 44-45. Branching of conidiophore x 540.

Fig. 46. Pure culture network x 1240.

Figure 44

Figure 45

Figure 46

they fuse to form simple 2-dimensional networks. The conidiophores are shorter than in pure culture. They reach 200u in length and bear 5-15 conidia. The conidia are identical to those in pure culture.

The distinguishing features of this species are the loosely capitate heads composed of 5 to 15 conidia produced on long, simple or branched sterigmata and the simple horseshoe-like arches which compose the networks.

Material Studied: Unnumbered, from Centraalbureau voor Schimmelcultures, Baarn, Holland.

9. Arthrobotrys oligospora Fresenius, Beitrage zur Mykologie. 1850

Syn: Didymozoophaga oligospora Sopr. Microbiology (In Russian) 20:489-499. 1951.
Arthrobotrys superba Corda var oligospora Matr. Recherches sur le Development de Quelques Mucedinees. 1892.
Arthrobotrys rosea Massee, Journal of the Royal Microscopical Society 5:758-759. 1885.

A translation from German of the original description by Fresenius is as follows:

"Simple, hyaline, septate conidiophores arise from prostrate base, up to ½ mm. tall; many short, blunt, stalk-like sterigma going out from the tip hold the spores. Spores pear-shaped, 1/28 mm. long with a septum somewhere below the middle, and a short apiculum at the base; About a dozen spores sit on the conidiophore tip forming a head. Below the point of the last sterigmata formed on many conidiophores is another small cluster of spores. More rarely are there up to six groups on the length of the conidiophore.

On wet beechwood, decayed fruit and in earth in fungal habitats."

The following account is based on a study of two isolates of A. oligospora. In pure culture, one of these isolates (CBS) forms a pale yellow colony. Its mycelium is entirely prostrate but heavy conidiation gives it a velvety appearance. The second isolate (No. 106) has a white to cream colored colony with both prostrate and aerial mycelium. The aerial mycelium forms in tufts

and gives the culture a wool-like appearance. The mycelium is 1.5-3u wide, hyaline, septate, and branched. Both aerial and prostrate mycelium produce conidiophores which appear in less than a week. They bear 20-30 heads of conidia at well separated nodes (Fig. 53, 54) but develop no branches. They are 5-7u wide at the base, but taper to 2-4u at a height of 200-450u where the first conidial heads are formed. The tip of the conidiophores and the nodal areas are swollen (Fig. 51) to 4-6u and bear 5-15 conidia in a tight capitate head on wartlike sterigmata (Fig. 49). The conidia (Fig. 47, 48, 50, 52) are pyriform and are constricted at the septum. The top cell is notably swollen and the base is apiculiform. They are 16.8-29.4 (24.4)u long, 8.4-15.5 (13.6)u wide and contain a proximal cell which is 4.2-11.2 (8.7)u long. The ratio of the distal cell to the proximal cell is 2:1. One isolate did form adhesive networks frequently in fresh pure culture (Fig. 55).

In nematode infested culture, the mycelium is all prostrate and hyaline. It forms adhesive hyphal loops which fuse into networks (Fig. 56). The conidiophores usually form in clusters of 3-12. They develop 1-4 nodes of conidia. The conidia measure 15-25u long and 7-12u wide, which is slightly smaller than in pure culture. The distal cell is less swollen than in pure culture. However, they are still constricted at the septum and the proximal cell end is apiculate. Yellow spherical or cylindrical chlamydospores which form intercalary in the mycelium may be either single or in chains.

After Fresenius described this species in 1850, Massee described another in 1885 which he called _Arthrobotrys rosea_ (Fig. 57). Massee's description is as follows:

> "_Arthrobotrys rosea_ tufted; pale rose colour; fertile flocci erect, sparingly septate, with three to five swollen nodes at equal distances, each node bearing a globose head of conidia, conidia broadly obovate, uniseptate, slightly constricted at the septum, apical segment largest, base apiculate."

Fig. 47. Conidium x 1040.

Fig. 48. Conidia x 540.

Fig. 49. Head of conidia showing capitate arrangment x 540.

Fig. 50. Conidia x 1240.

Fig. 51. Conidiophore tip x 540.

Figure 47

Figure 48

Figure 49

Figure 50

Figure 51

Fig. 52. Conidium x 1600.

Fig. 53. Nodular development of conidiophore x 540.

Fig. 54. Nodular development of conidiophore x 1240.

Figure 52

Figure 53

Figure 54

Fig. 55. Pure culture networks x 1240.

Fig. 56. Nematode in network x 1240.

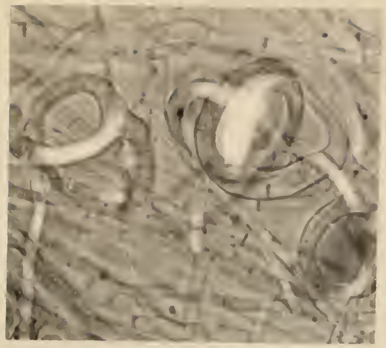

Figure 55

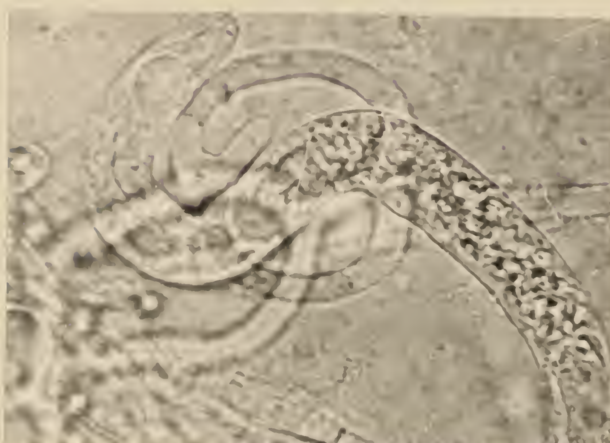

Figure 56

Massee's original illustration is reproduced in Fig. 57.

This species was first considered a synonym of A. oligospora by Matruchot (1863). Drechsler (1937) also studied it and suggested that it should be put into synonomy. His reasons were that this species is nodular and has obovate conidia which are constricted at the septa. Also the illustration depicts the same proportions between the basal and distal cells as is given for A. oligospora. Because of this, it is listed here as a synonym.

Material Studied: Unnumbered, from Centraalbureau voor Schimmelcultures, Baarn, Holland; No. 106, from mushroom bed, Kansas City, Mo., Richard Haard, 1964.

10. Arthrobotrys oviformis Sopr., Predaceous fungi-Hyphomycetes and their application in the control of pathogenic nematodes. (In Russian). 1958.

Syn: Didymozoophaga oviformis Sopr. Microbiology (In Russian) 20:489-499. 1951.

A translation from Latin of the original description by Soprunov is as follows:

"Colony widely spread, expanding, loosely weblike; sterile hyphae prostrate, branched, septate, hyaline, mostly 3-5u wide; trapping loops around 30u in diameter, circular or arch-like, the network adhesive. Nematodes ensnared. Conidiophores erect, septate, branched, tip and along conidiophore nodular - with sterigmata, hyaline sterigmata minute, 4-6 together; fertile hyphae 200-450u tall, base 6.5-9u wide gradually narrowing toward the top. Conidia hyaline, obovoid, constricting at septum, uniseptate, two cell always unequal, superior cell globose and always larger, smaller cell proximal, tip of the proximal cell apiculiform, conidia 26.5u long (22.5-32.5), 13u wide (10-15). Chlamydospores round, 15-30u in diameter."

This species (Fig. 58) appears related most closely to A. arthrobotryoides and A. dolioformis. All three species have branched and nodular conidiophores. However, A. oviformis alone has obovoid conidia which are constricted at the septum. Material was unavailable for study.

Fig. 57. _A_. _rosea_. Reproduced from Massee(1885).

Figure 57

Fig. 58. A. *oviformis*. Reproduced from Soprunov(1958).

Figure 58

11. Arthrobotrys robusta Duddington, Trans. Brit. Mycol. Soc. 34:598-600. 1951.

Syn: Trichothecium robustum Sopr., Predaceous fungi-Hyphomycetes and their application in the control of pathogenic nematodes. (In Russian). 1951.

A translation from Latin of the original description by Duddington is as follows:

"Vegetative hyphae straight, septate, 2-7u wide, partly branched, forming firm networks which seize nematodes; hypha enters through integument of the nematode to form infection bulb. Conidia hyaline, oblong, pyriform, uniseptate, 18-27u long, 8-12u wide. Conidiophores erect, sometimes branched, 150-300u tall altogether.

Capturing and consuming nematodes in rotten wood around Mickleham, Surrey, England. Oct. 1948."

In his discussion, Duddington included additional pertinent details including some differences between pure and nematode infested cultures. He reported that the mycelium was 2-7u in diameter and in nematode infested cultures produced three dimensional networks. These networks, which were composed of loops 20-60u in outside diameter, captured nematodes by adhesion. The conidia, which were larger than in pure culture, formed early and in great profusion. They were produced in a capitate head at the apex of each branch of the conidiophore which lacked nodal development.

The following account is based on the study of one isolate. Although this isolate differs in some respects from Duddington's description, it seems to be within the limits of this species.

The colony in pure culture is white, irregularly rounded, and cushion-like. It contains both prostrate and aerial mycelium. The aerial mycelium is suberect and often runs laterally above the substratum. Because it entangles and fuses, it forms the cushion-like structure. Both types of mycelia are hyaline, septate, highly branched, 2-7u wide, and produce a profusion of conidiophores. The first of the conidiophores comes into evidence in less than a week after

inoculation. The conidiophores range from 100-500u in length with most of them found between 200-300u. They are 4-6.5u wide at the base and taper to 2-4u just below the head. Most of the conidiophores are highly branched especially near the top. (Fig. 66). They form a capitate head of conidia at the apex of each branch. Five to fifteen conidia are borne on long sterigmata on the nonswollen tip of the conidiophore (Fig. 61, 62, 65) which rarely elongates to form more than one node. However, when this does occur, only a few conidia form at the tip before the conidiophore elongates to form a normal terminal head (Fig. 63, 64, 65). The conidia (Fig. 59, 60) are uniseptate, elongate ovoid to elongate obovoid, and have a definite pedicel. They are 21-40 (30.7)u long, 7-12.5 (9.7)u wide and contain a proximal cell 9.8-24 (17.5)u long. The ratio of the two cell lengths is approximately 1:1 with the distal cell slightly swollen. The septum does not constrict. Chlamydospores were not observed.

As yet, this isolate has not been grown in nematode infested culture. It has died every time nematodes were introduced into a culture.

This isolate varies from Duddington's in the size and shape of the conidia, but other characteristics agree with those of the description.

Material Studied: No. 8416, from culture collection, U.S. Army Natick Laboratories.

12. Arthrobotrys superba Corda, Pracht-Flora Europaeischer Schimmelbildungen. 1839.

Syn: Didymozoophaga superba Sopr., Microbiology (In Russian) 20:489-499. 1951.
Arthrobotrys drechsleri Sopr., Predaceous fungi-Hyphomycetes and their application in the control of pathogenic nematodes. (In Russian). 1958.

A translation from Latin of the original description by Corda is as follows:

Fig. 59. Conidia x 540.

Fig. 60. Conidium x 1280.

Fig. 61. Conidiophore x 540.

Fig. 62. Sterigmata x 540.

Figure 59

Figure 60

Figure 61

Figure 62

Figs. 63-64. Nodular development of conidiophores x 540.

Fig. 65. Sterigmata x 580.

Fig. 66. Branching of conidiophore x 540.

Figure 63

Figure 64

Figure 65

Figure 66

> "Colony white-glistening, conidiophores simple and hyaline, spore head sphere-like and swollen; spores oblong, top broadly rounded, medium constricted, base pointed, white."

The present study is based on three specimens of A. superba. In two of the colonies (No. 7365 and 123), aerial mycelium rises from the prostrate hyphae in small tufts producing a "wool-like" appearance. Both of these colonies spread in a circular pattern and leave the center of the colony bare of aerial mycelium. One of these colonies (No. 123) is white and the other (No. 7365) is rose colored. In the third colony (No. 1688) the white aerial mycelium spreads rapidly over the plate and often intertwines with itself to form a cushion-like effect.

The mycelium is septate, hyaline, branched, and 1.5-5.5u wide. Conidiophores appear in 1-2 weeks from both the aerial and the prostrate mycelium. They grow to a length of 50-200u if they are produced from aerial mycelium or 200-500u if produced from prostrate mycelium. The conidiophores are 3-5.5u wide at the base and taper to 1.5-4u below the swollen tip which is 4.5-8.5u wide. The conidiophores are unbranched (Fig. 71), but they are nodular and often elongate past the first head to form 20 or more capitate heads of conidia (Fig. 73, 74). Each node is swollen (Fig. 70) and produces 5-15 conidia on short wartlike sterigmata in a whorled pattern (Fig. 69, 72). This heavy production of conidia often causes the conidiophores to bend. The conidia (Fig. 67, 68) are uniseptate, hyaline, oblong ovoid, broadly rounded at the distal end, tapered slightly at the proximal end and terminated in a short apex. The conidia used in this study are less constricted at the septum and less pointed at the apex than those illustrated by Corda. The conidia are 16.8-26.5 (21)u long, 5.6-11.2 (8.2)u wide with a proximal cell 8.4-12.6 (10)u long. The ratio of the distal cell length to the proximal cell length is 1:1.

Fig. 67. Conidia x 1240.

Fig. 68. Conidia x 800.

Fig. 69. Head of conidia showing capitate arrangment x 540.

Fig. 70. Conidiophore tips x 540.

Figure 67

Figure 68

Figure 69

Figure 70

Fig. 71. Conidiophores x 125.

Fig. 72. Conidiophore tip x 1240.

Fig. 73-74. Nodular development of conidiophores x 540.

Figure 71

Figure 72

Figure 73

Figure 74

In nematode infested cultures, A. superba has only prostrate mycelium. It forms adhesive loops which are often compounded into networks. The conidiophores are erect, 100-300u long, 3-5u wide at the base, 2-3.5u wide at the tip, and unbranched. In most cases, only a single terminal head of 4-10 conidia is produced. However, occasionally 2 nodes are formed. Three nodes on a single conidiophore have not been found. The size of conidia is essentially the same as in pure culture. Drechsler (1937) reported finding an apothecium in nematode infested culture but was not able to maintain it long enough to obtain a culture from it.

Material Studied: No. 1688 and 7365, from culture collection, U.S. Army Natick Laboratories; No. 123, from decaying wood, Wildcat Creek, Manhattan, Riley Co., Kansas, Karen Haard, April 1963.

Excluded Species

The species listed in this section are excluded from the previous section for one or more of the following reasons: 1) their descriptions lack details necessary for accurate identification of material; 2) appear synonymous with established species; or, 3) differ from other species to the extent that they probably belong in other genera. Material, including the type specimens of these species, was unavailable at this time.

In the genus Arthrobotrys, certain characteristics are needed to define a species. The size, branching, nodular development, and type of tip of the conidiophore should be included as well as the size, shape, and the ratio of distal cell length to proximal cell length in the conidium. In addition, any indication of predaceous activity as well as the type of predaceous organs needs to be ascertained. In the majority of the species that follow, the

original descriptions are too brief and fail to note some of the diagnostic characteristics needed to allow one to confidently place them among the previously discussed species.

1. Arthrobotrys deflectans Bresadola Annales Mycologica 1:2, 128. 1903.

A translation from Latin of the original description by Bresadola is as follows:

"Broadly spreading, dry loosely entwined hyphae, white; vegetative hyphae non-erect; conidiophores erect, septate, varying in length, 2.5-3u wide, with septate, whorled conidiophores, non-nodular; conidia sessile, hyaline, uniseptate, subfusoid, straight or slightly curved, 10-18 x 2-2.5u.

Habitat: on trunk Pinus silvestris."

This species differs from the other members of this genus as it has extremely narrow subfusoid conidia and lacks sterigmata. The description, however, lacks information on the size and branching of conidiophore, the type of conidiophore tip, and the presence and type of predaceous organs. Because it has not been recorded or verified since the original collection and because the description both lacks details and differs substantially from other species it is probably best to consider this species a nomen dubium.

2. Arthrobotrys kirghizica Soprunov, Predaceous fungi-Hyphomycetes and their application in the control of pathogenic nematodes. (In Russian). 1958.

Syn: Didymozoophaga kirghizica Soprunov, Microbiology (In Russian) 20:6, 489-499. 1951.

A translation from Latin of the original description by Soprunov is as follows:

"Colony widely spreading, grey-white; vegetative hyphae non-erect, branched, septate, hyaline, 2-6u wide, trapping loops frequently around 25u diameter, circular or arch-like, frequently

forming together in networks, the adhesive loops ensnaring nematodes. Conidiophores erect, septate, hyaline, branched one-way, around 300-500u tall, base 5-7u wide, tapering upward, apex and along length of conidiophore wider with sterigmata-bearing nodes; conidia hyaline, ovoid, uniseptate with septum slightly or non-constricting, distal apex widely rounded, proximal apex gradually tapering, 21.6u long (18.5-24.5), 10.5u wide (9-12.5), resting spores around 12-15u in diameter. Capturing and consuming nematodes.

Habitat in earth around Askhabad, Turkmenia. U.S.S.R."

This species (Fig. 75) described by Soprunov appears to be synonymous with A. arthrobotryoides (Berlese) Lindau. Both species have branched nodular conidiophores, swollen conidiophore tips, short sterigmata, and conidia of the same size and shape. Soprunov, however, gives a larger measurement for his conidiophore and a slightly different mode of branching.

3. Arthrobotrys longispora Pr., Linnaea 26:708. 1853.

A translation from Latin of the original description by Preuss is as follows:

"Colony spreading, white; vegetative mycelium prostrate, septate, hyaline; conidiophores suberect, sometimes branched, tip inflated whorl; spores uniseptate, oblong, base apiculiform; outer spore coat white; nucleus granular."

The information in this description is too incomplete to positively distinguish a species of this genus. It lacks information on the nodular development and size of conidiophores, the size and shape of the spores and the sterigmata. In addition, no mention of predaceous organs was made, however, the predaceous activity of this genus was not known at that time. The suberect branched conidiophores with inflated tips does indicate the possibility that this species does belong in Arthrobotrys and that it may be closely related to A. cladodes.

Soprunov redescribed this species (Fig. 76) in 1958; however, because type material is no longer existent and because of the lack of information in

Fig. 75. *A. kirghizica*. Reproduced from Soprunov(1958).

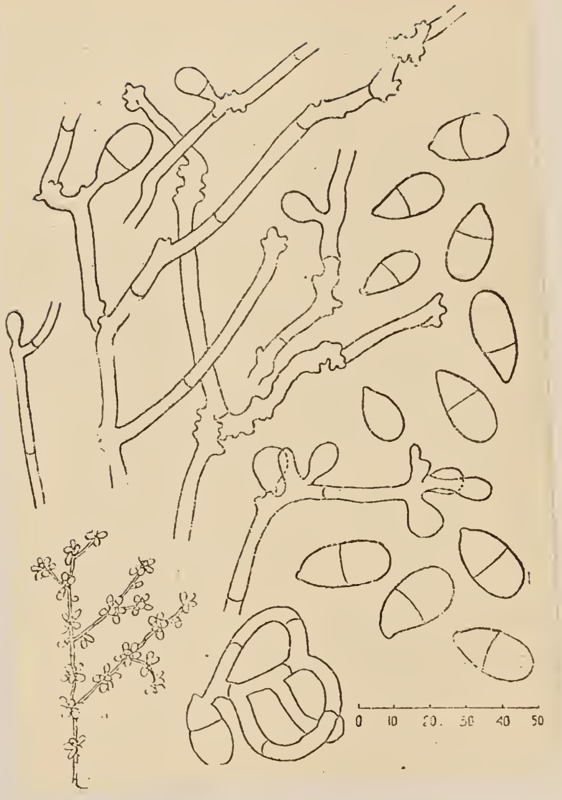

Figure 75

Fig. 76. _A_. _longispora_. Reproduced from Soprunov(1958)

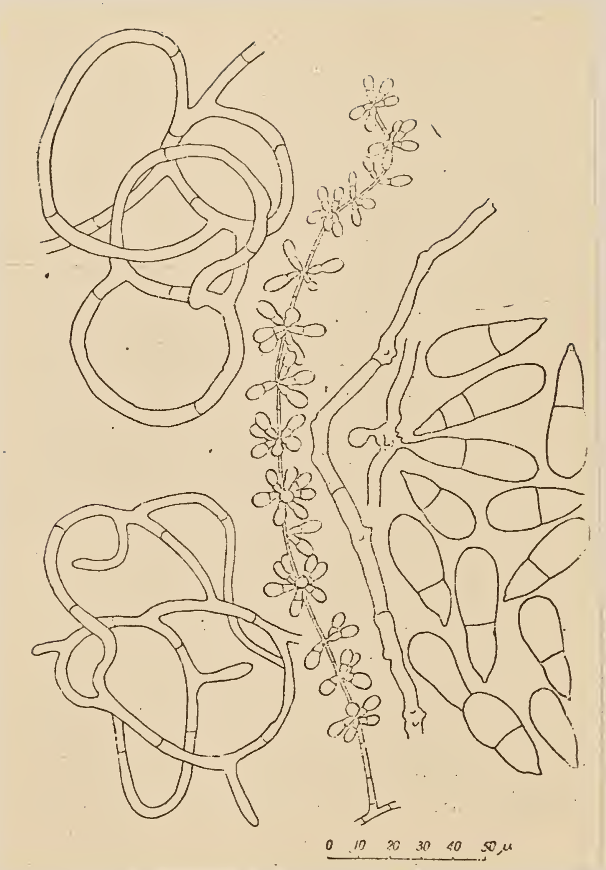

Figure 76

the original description there is no way of knowing whether or not he was dealing with the same organism as Preuss. A translation from Latin of this description of Soprunov is as follows:

> "Colony cushion-like, first white, then rosy yellow; vegetative hyphae nonerect, branched, septate, hyaline, 2-6u wide, trapping loops around 60u diameter, circular or archlike, sometimes joining to form networks, the adhesive loops ensnaring nematodes; conidiophores erect, simple, straight, or bending, tip inflated whorl, around 400-800u tall, base 6-8u wide, tapering upward, conidia born on sterigmata, oblong, uniseptate, constricted at septum, two cells unequal, proximal cell smaller, 38.5u long (23-45.5), 13.2u wide (10.5-16.5).
>
> Habitat in earth and in fallen tree branches around Askhabad, Turkmenia. U.S.S.R."

Discrepancies can be noted between Soprunov's description and the original by Preuss. Preuss states that the conidiophores are branched and the conidia oblong while Soprunov states that the conidiophores are unbranched and nodular and the conidia obovoid.

Soprunov's description and illustration would better seem to fit the species <u>A. conoides</u> Dr. Both Soprunov's species and <u>A. conoides</u> have nodular unbranched conidiophores, ovoid conidia which are constricted at the septum and predaceous networks.

4. <u>Arthrobotrys</u> <u>recta</u> Pr., Linnaea <u>24</u>:128. 1851.

A translation from Latin of the original description by Preuss is as follows:

> "Mycelium spreading, white, vegetative hyphae wool-like, nonerect, septate; hyaline, erect, septate conidiophores, tip an expanded whorl; spores uniseptate, obovoid, base provided with an apiculiform hylum; spore coat transparent white, nucleus granular.
>
> Habitat in semi-rotten <u>Brassicae</u> <u>oleraceae</u>, Hoyerswerda."

Matruchot (1863) suggested that this species was synonymous with _A. oligospora_ Fresen. because it had obovoid spores. He apparently had not seen material of this species and thus based his conclusion on the description. However, since this species has not been reported since 1851 and so little information is given in the description, it seems best to classify it as a _nomen dubium_.

5. _Arthrobotrys straminicola_ Pidoplichko, Mikrobiologichnii Zhurnal _9_:55. 1948.

A translation from Latin of the original description by Pedoplichko is as follows:

> "Colony dirty yellow. Conidiophores sparingly septate, 6-10 swollen vesicles, nodes equal distance from apex, head globose provided with conidia. Conidia oblong ellipsoidal, 10-17 x 4.4-6.5u, uniseptate, the septa nonconstricting, apex blunt, often near base gradually abbreviated.
>
> In spikes, seeds, and stalks of wet _Tritici vulgaris_ and _Avenae sativae_. Baschcortostan (prov. Ufa). U.S.S.R."

A. straminicola (Fig. 77) may be a good and valid species of _Arthrobotrys_ but it is excluded from the list of species because the description and illustration omit details on the conidiophore size and branching, size of cells of conidia and predaceous activity. Also, type material of this species has been unobtainable at this time. Pedoplichko published additional information on this species in 1953, however, this too has been unobtainable.

6. _Arthrobotrys stilbacea_ Meyer Bull. Soc. Mycol. Fr. _74_:246. 1958.

A translation from Latin of the original description by Meyer is as follows:

> "In nature: form Stilbaceae:
>
> Coremia cream colored, 2-3mm tall, cylindrical base; the upper half of the cylinder frequently forming a fertile cone. Sporogenous

Fig. 77. A. straminicola. Reproduced from Pedoplichko(1944).

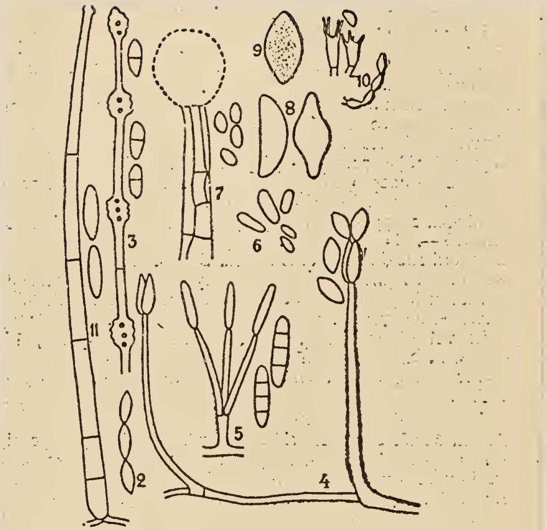

Рис. 2. *1*—Fusidium flagelliforme (конідіофор і конідії); *2*—F. elegantulum (конідії); *3*—Arthrobotrys straminicola (вершечок конідіофора і конідії); *4*—Hyalobotrys elegans (конідіофори і конідії); *5*—Dactylium olivascens (розгалуження конідіофора і конідії); *6*—Graphium guttuliferum (конідії); *7*—Graphium minutellum (коремій і конідії); *8*—Melanospora lunulata (аскоспори); *9*—Melanospora asperrima (аскоспора); *10*—Corticium straminicola (базидії, конідія і частка гіфи) (×700; 3—×450).

Figure 77

cells hyaline, free of coremia at 30-50, extremity generally inflated with numerous denticles which bud forth spores. Conidia becellular, hyaline, obovoid to cylindroid, 10.5-14 x 4-4.5, produced dry from the end cell of the sporogenous tip.

In culture: form Moniliaceae:

Colony white, thin, spreading. Conidiophores simple, hyaline, lengthening to 200u long and 3-5u in diameter, 1-4 delaying sporiferous teeth bud forth. Conidia hyaline, 2-celled, obovoid to cylindroed, 12-16u x 4-5u."

This species (Fig. 78) differs from the other species in Arthrobotrys in two major ways. First, no other species has been reported having a stilbaceous form. This factor indicates that this species should belong in the form family Stilbellaceae under the Saccardian system of classification. The genus Arthrosporium (Fig. 79) Sacc. in this family appears to resemble closely A. stilbacea. Both have synnemata; both have conidiophore elongation; and both bear conidia on small sterigmata on swollen conidiophore tips. But the conidia of Arthrosporium have three septa while those of A. stilbacea have only one. There appears to be a relationship between Arthrosporium and A. stilbacea similar to that between Arthrobotrys and Dactylaria. Thus, it seems that it may be necessary to erect a new genus in the Stilbellaceae to embrace A. stilbacea.

Another possibility in classifying A. stilbaceae is suggested by the classification of such pleomorphic forms as the Cephalosporium and Graphium imperfect stages of Ceratocystis ulmi. The stages could be divided with the moniliaceous stage left in Arthrobotrys while the stilbaceous stage be removed to a new genus in the Stilbellaceae. However, one fact should be clarified before such a step is taken. Meyer isolated the moniliaceous form by inoculating plates with the stilbaceous form. However, he did not report inoculating the moniliaceous form back onto the palm leaf and reisolating the

Fig. 78. A. stilbacea. Reproduced from Meyer(1958).

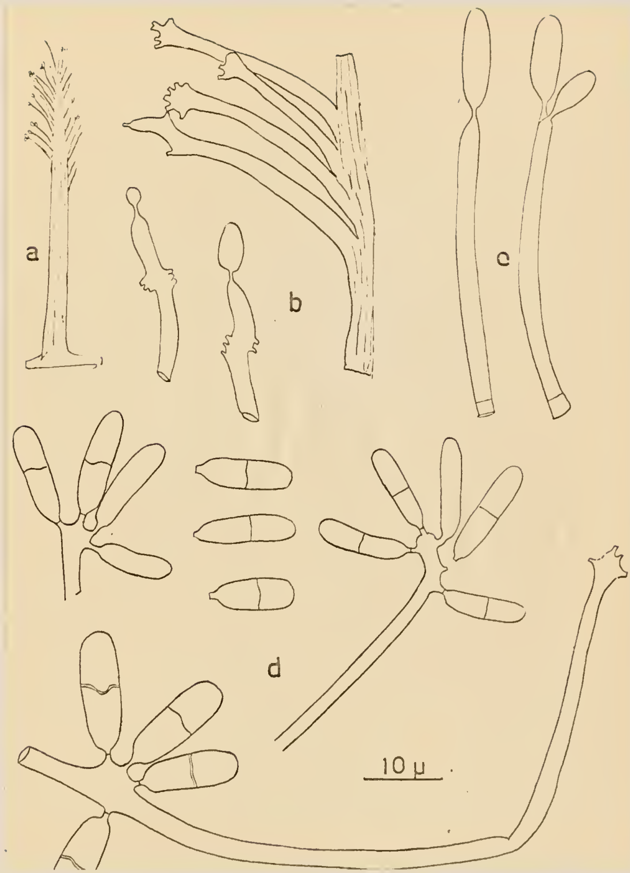

Figure 78

Fig. 79. Arthrosporium sp. Reproduced from Morris(1963).

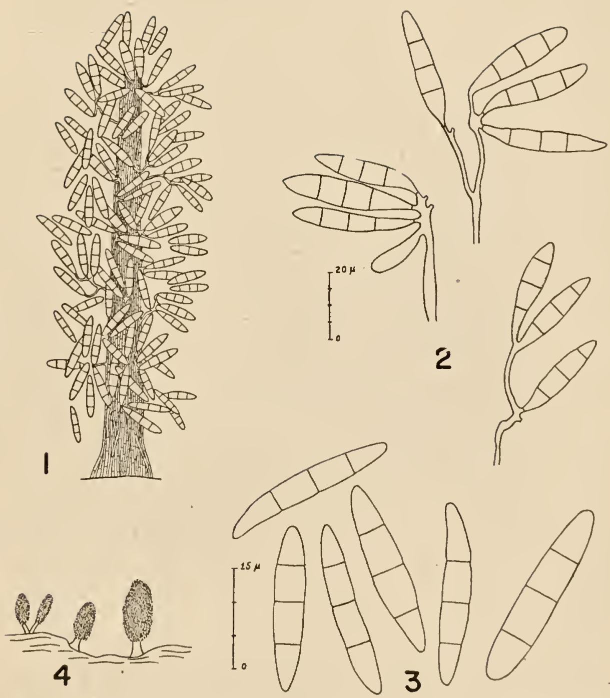

Figure 79

stilbaceous form. This leaves the possibility that the moniliaceous form could be a contaminant derived from the foliar material upon which the synematous form was parasitic.

This points to the second major difference between A. stilbacea and the other species of Arthrobotrys. A. stilbacea is recorded as a plant parasite while all of the other species of Arthrobotrys are predators on nematodes except one which is a predator on springtails. None of these species have been reported as being parasitic on plants.

For these reasons, A. stilbacea is included in this section until more is known about this organism and a more sound decision can be made regarding its taxonomy.

7. Arthrobotrys strangulans Maupas

This species is illegitimate as it has no published description.

DISCUSSION

The concept of the genus Arthrobotrys has evolved in great detail since it was first described 125 years ago. Although the original description stated that the conidia were born solitary on knot-like sterigmata on the swollen nodes of simple, erect conidiophores, the genus now contains species with non-nodular or branched conidiophores that have nonswollen tips and bear conidia on long sterigmata.

Twelve species of Arthrobotrys are now recognized as being valid. When they are grown in pure culture on maizemeal agar, these species break down into four distinct groups as outlined in Table 3.

Table 3

Major Groups Within the Genus Arthrobotrys

Group	Characteristics: Conidiophores	Characteristics: Predaceous Organs	Species
I	Branched, non-nodular	3-D networks	A. cladodes A. robusta
II	Branched, nodular	3-D networks	A. arthrobotryoides A. dolioformis A. oviformis
IIIa	Unbranched, non-nodular	2-D networks	A. musiformus
b	Unbranched, non-nodular	Constricting rings	A. anchonia A. dactyloides
IVa	Unbranched, nodular	3-D networks	A. conoides A. oligospora A. superba
b	Unbranched, nodular	Aerial predaceous organs	A. entomopaga

These groups (Table 3) into which the species of Arthrobotrys have been divided, indicate some interesting trends of development in the conidiophores and the predaceous organs within this genus. The simplest among the species appears to be those listed in Group I. These species have conidiophores that are little more than erect or suberect vegetative hyphae which have differentiated to the point that each branch bears a terminal head of conidia. The branched conidiophores develop either a swollen conidiophore tip with "wart-like" sterigmata or a nonswollen tip with short "branch-like" sterigmata. All of the conidia in this group are slightly elongate obovoid, contain two cells which are equal in length, and are not constricted at the septum. However, they vary greatly in size. All members within this group form predaceous networks in nematode infested cultures. These are composed of fused vegetative hyphae which have differentiated to the point that they are covered with an adhesive substance. Two species, A. cladodes and A. robusta are at present within this initial group.

The second group as outlined in Table 3 contains three species that are similar to those in Group I in having branched conidiophores and network-like predaceous organs. They differ, however, in having a nodular development of the conidiophores which appears to be a definite step away from the vegatative growth habit. The conidiophores in all species within this group have a swollen tip and bear their conidia on short "wart-like" sterigmata. On species (A. arthrobotryoides) in this group varies at times and produces its conidia on long sterigmata in a rachis-type arrangement on the conidiophore. The conidia produced within this group vary widely in both size and shape. They may be ovoid, slightly obovoid, strongly obovoid, or elongate obovoid. They may or may not be constricted at the septum, and the two cells may be equal or unequal in length. All species in this group develop 3-dimensional networks like those of the preceding group. Three species, A. arthrobotryoides, A. dolioformis, and A. oviformis are represented in this group.

Group III as outlined in Table 3 contains three species which have unbranched, non-nodular conidiophores which appear to have lost the ability to branch. They have nonswollen tips and short "branch-like" sterigmata. The conidia in this group vary from obovoid to elongate ellipsoid. They are not constricted at the septa, and the two cells in them may be either equal or unequal in length.

Two of the species in this group, A. anchonia and A. dactyloides appear to have undergone a high degree of specialization in the development of their predaceous organs. They have three-celled constricting rings on short stalks which are entirely nonadhesive. The third species, A. musiformis, has a type of predaceous organ that appears to be intermediate between the 3D-networks and the constricting rings. They are adhesive 4-celled loops which are both singly, directly on the mycelium or fused together in a 2-D network.

Group II appears to be a link between Group I and Group IV which has nodular, unbranched conidiophores. The conidiophores in this as in Group III appear to have lost the ability to form branches. This seems to be another definite step away from the vegetative habit. Also the conidiophores in all species of this group develop swollen tips which bear short "wart-like" or "branch-like" sterigmata. The conidia here also vary in size and shape being obconical, pyriform, or ovoid. The two cells of the conidia are either equal or unequal in length and there may or may not be a constriction at the septum.

Three of the species of this group, A. conoides, A. oligospora, and A. superba form 3-D networks similar to those of Groups I and II. However, A. entomopaga seems to be somewhat removed from the other three species in that its networks have undergone considerable specialization for the capture of springtails rather than nematodes. This is the only species of the genus which is predaceous on springtails, all others capture nematodes. These specialized aerial organs arise at the junction of fused hyphae and consist of two elongated cells of which the distal one secretes a sticky material that entraps springtails by adhesion.

All of these groups differentiate in nematode infested cultures. The conidiophores which are normally branched in pure culture do not form branches in nematode infested cultures. Those that are nodular in pure culture either form fewer nodes or lack them completely in nematode infested cultures. The colonies in all species also undergo a change and produce only prostrate mycelium in nematode infested cultures.

Perhaps the most striking difference between pure and nematode infested cultures is the fact that these species all produce predaceous organs when nematodes are present. This difference can be erroneous. Two isolates of

different species from Group IV, A. conoides and A. oligospora, readily produced networks in pure culture within two weeks of inoculation. The only other instance reported of this is one by Pramer (1964) when he found a few networks of A. conoides in pure culture. The reason they formed readily in pure culture in these instances is unknown.

One species from Group III has been observed to form constricting rings in pure culture. This differs from the incidents above because these formed only in old desiccated cultures that had been refrigerated for one year. One other species from Group III has in one isolated incident been observed to form networks in old desiccated petri plates. This species, A. musiformis, formed a few 2-D networks in one petri dish culture that was over 2 months old. These incidents may have been caused by the lack of certain nutrients in the old cultures. This has not been previously reported.

The exact manner of conidiophore development has also been studied. Although Hughes (1953) suggested that the manner of conidiophore development in Arthrobotrys might properly fit into his Section II, enough differences have been observed to perhaps warrant erecting a new section to embrace this genus. In Hughes Section II, the conidiophore proper elongated after the production of each conidium and the second conidium was always formed above the first. In Arthrobotrys, the conidiophore elongates in the form of a sterigmata, slightly below and immediately to the side of the first conidium produced. Thus the second conidium forms below the first instead of above it.

The part that nuclei play in conidium development in Arthrobotrys also differs from the example given by Hughes for his Section II. In Hughes' example, a single nucleus enters the developing conidiophore and remains at the base. When a conidium begins to form, the nucleus divides and one

daughter nucleus travels up the conidiophore and into the conidium. In Arthrobotrys, several nuclei are present in the young conidiophore and become located in the tip. As a young conidium is being formed, one or more are forced into the developing conidium with the influx of cytoplasm.

CONCLUSIONS

The genus Arthrobotrys contains some 20 names of species that have been included as members of the genus. At the present time, only 12 of these appear to be valid species. The characteristics which bind these species together and at the same time separate them from those of closely related genera are the simple erect conidiophores with a terminal head of conidia, the uniseptate conidia on sterigmata and the whorled pattern of arrangement of conidia on the conidiophore.

Much of the confusion which has arisen regarding the species of this genus apparently stems from the fact that they vary greatly in morphology when grown in nematode infested culture and in pure culture. When members of this genus are grown in nematode infested culture, those conidiophores often do not form branches and either do not form nodes or produce them in limited numbers. They also produce predaceous organs in greater profusion than in pure culture.

A given species appears to be considerably more stable when grown in pure culture and thus emphasis has been placed in studies of these organisms in pure culture. When members of this genus are grown in pure culture, there appears to be four distinct groups which are separated primarity on the basis on the conidiophore characteristics. The characteristics of each group are summarized in Table 3.

All species in this genus produce conidia in the same manner whether in nematode or pure culture. Each conidiophore as it develops has several nuclei located in its tip. The first conidium forms terminally on the conidiophore. As it develops, an influx of cytoplasm from the conidiophore tip carries several nuclei into it. The conidiophore then elongates as a sterigmata just below and to the side of the first conidium formed and produces a second in the same manner. Thus every conidium is produced slightly below the last one.

SUMMARY

Studies on the genus Arthrobotrys have been undertaken using twenty-one isolates of eight species. Eleven of these isolates were obtained from the U.S. Army Natick Laboratories Stock Culture Collection and Centraalbureau voor Schimmelcultures, Baarn, Holland. The remaining ten were isolated from soil and decaying organic matter.

These isolates were studied in pure culture from maizemeal plates, slide cultures, and mounted preparations, and in nematode infested culture from maizemeal plates. The nuclear conidition was studied from permanent mounts stained with Azure A.

From this study, twelve species were concluded to be valid. Two keys were then prepared for the separation and identification of these twelve species. The first was prepared for use in identifying the species when they were grown in pure culture on maizemeal agar, and the second for use in identifying species in nematode infested cultures on maizemeal agar plates.

These twelve species may be divided into four distinct groups on the basis of the characteristics of their conidiophores in pure culture and their predaceous organs. Group I has branched non-nodular conidiophores and 3-D

networks. Group II has branched nodular conidiophores and 3-D networks. Group III has unbranched non-nodular conidiophores and either 2-D networks or constricting rings. Group IV has unbranched nodular conidiophores and either 3-D networks or aerial predaceous organs. However, in nematode infested cultures, conidiophores may or may not branch and form nodes.

All species in this genus, however, produced their conidia in the same manner regardless of whether they were grown in pure or nematode infested cultures. Several nuclei became located in the tip of the developing conidiophore. The conidiophore formed its first conidium terminally, and as it formed, several nuclei were carried into it with the influx of cytoplasm. After the first conidium was formed, the conidiophore elongated from just below and to the side of it forming a sterigmata on which the second conidium was formed in the same manner. Up to 30 conidia may be produced, each below the last on the conidiophore head.

Eight species listed previously as members of the genus Arthrobotrys were concluded to be of doubtful validity.

INDEX TO SPECIES

LITERATURE CITED

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STUDIES ON THE GENUS
ARTHROBOTRYS

by

KAREN KAYE HAARD

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Major Professor

The genus Arthrobotrys is found amid the predaceous Hyphomycetes of the Fungi Imperfecti. It has received considerable attention because of its predaceous activity, however, little has been done with it taxonomically. A comprehensive treatment of this genus is needed since many of the species have been confused. It is hoped that the present study will help clarify this situation.

Studies on the genus Arthrobotrys were done from the literature and twenty-one isolates of eight species. Eleven of these isolates were obtained from the U.S. Army Natick Laboratories Stock Culture Collection and Centraalbureau voor Schimmelcultures, Baarn, Holland. The remaining ten were isolated from soil and decaying organic matter.

These isolates were studied in pure culture from maizemeal plates, slide cultures, and mounted preparations, and in nematode infested culture from maizemeal plates. The nuclear condition was studied from permanent mounts stained with Azure A.

From this study, twelve species were concluded to be valid. Two keys were then prepared for the separation and identification of these twelve species. The first was prepared for use in identifying the species when they are grown in pure culture on maizemeal agar, and the second for use with cultures isolated in nematode infested cultures on maizemeal agar plates.

These twelve species may be divided into four distinct groups according to their conidiophore characteristics when they are grown in pure culture on maizemeal agar and their predaceous organs. These groups are outlined in the following table.

Major Groups Within the Genus *Arthrobotrys*

Group	Characteristics: Conidiophores	Characteristics: Predaceous Organs	Species
I	Branched, non-nodular	3-D networks	A. cladodes A. robusta
II	Branched, nodular	3-D networks	A. arthrobotryoides A. dolioformis A. oviformis
IIIa	Unbranched, non-nodular	2-D networks	A. musiformis
b	Unbranched, non-nodular	Constricting rings	A. anchonia A. dactyloides
IVa	Unbranched, nodular	3-D networks	A. conoides A. oligospora A. superba
b	Unbranched, nodular	Aerial predaceous organs	A. entomopaga

When these species were grown in nematode infested culture, they lost or became limited in their ability to branch or form nodes.

All species in this genus, however, produced their conidia in the same manner regardless of whether they were grown in pure or nematode infested cultures. Several nuclei became located in the tip of the developing conidiophore. When the conidiophore formed its first conidium, several nuclei were carried into it with the influx of cytoplasm. After the first conidium was formed, the conidiophore elongated from just below and to the side of it forming a sterigmata on which the second conidium was formed. Up to 30 conidia may be formed in this fashion, each below the last on the conidiophore tip.

Eight species listed previously as members of the genus *Arthrobotrys* were concluded to be of doubt validity.